COMBINATION OF H1, H3 AND H4 RECEPTOR ANTAGONISTS FOR TREATMENT OF ALLERGIC AND NON-ALLERGIC PULMONARY INFLAMMATION, CONGESTION AND ALLERGIC RHINITIS

This application claims the benefit of U.S. Provisional Patent Application No. 60/443,207, filed January 28, 2003, which is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention provides methods for treating allergic, non-allergic pulmonary and nasal obstructive disease conditions by administration of histamine receptor antagonist combinations.

BACKGROUND OF THE INVENTION

Allergic rhinitis, pulmonary inflammation and congestion are medical conditions which may be associated with other medical conditions including asthma, chronic obstructive pulmonary disease (COPD), seasonal allergic rhinitis and perennial allergic rhinitis. In general, these conditions are mediated, at least in part, by inflammation which may be controlled by antagonizing histamine receptors.

Allergic rhinitis, sometime referred to as "hay fever", is a common illness affecting an estimated 20-40 million Americans, and resulting in 10 million lost days of school or work each year. Two types of allergic rhinitis include seasonal allergic rhinitis and perennial allergic rhinitis. Similarly, congestion, particularly sinus congestion is characterized by inflammation of the tissues in the sinus cavities. Common remedies for rhinitis are "antihistamine" H1 receptor antagonists such as chlorpheniramine maleate.

COPD, asthma and repeated episodes of pulmonary inflammation can lead to alveolar damage and fibrosis which can lead to impaired lung capacity and gas exchange. In general, exposure of the lungs to allergens may lead to mast cell mediated release of histamine and other substances which, in turn, begins a cascade of events leading to inflammation.

U.S. Patent Nos. 5,217,986 and 5,352,707 to Pomponi *et al.* attribute an ability for treating conditions including rhinitis and airway congestion to certain compounds

apparently having H3 receptor binding activity, but no H1 receptor antagonist activity. No clinical observation or other support is provided for this proposition.

International Patent Application Publication No. WO 02/56871 discloses, generally, the use of a combination of histamine H1 and H4 receptor antagonists for treating allergic disorders and diseases. The publication does not, however, exemplify any particular H1 or H4 receptor antagonists which may be useful for this purpose.

International Patent Application Publication No. WO 98/06394 discloses the use of a combination of histamine H1 and H3 receptor antagonists for treating upper airway allergic responses. The Application does not disclose use of a histamine H4 receptor antagonist.

Further, U.S. Patent No 5,869,479 discloses compositions for the treatment of the symptoms of allergic rhinitis using a combination of at least one histamine H1 receptor antagonist and at least one histamine H3 receptor antagonist. The patent does not mention use of an histamine H4 receptor antagonist.

Accordingly, there is a need in the art for an effective method by which to treat or prevent medical conditions such as allergic rhinitis, pulmonary inflammation and congestion by antagonizing histamine receptors such as H1, H3 and H4.

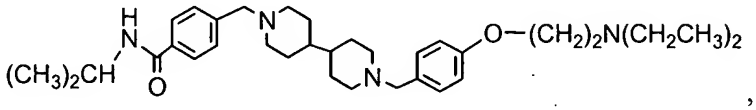
SUMMARY OF THE INVENTION

The present invention provides a method for treating or preventing an allergic or non-allergic condition characterized by airway inflammation (*e.g.*, allergic rhinitis, congestion or pulmonary inflammation) in a subject (*e.g.*, a human) comprising administering one or more histamine H3 receptor antagonists, one or more histamine H4 receptor antagonists and, optionally, one or more histamine H1 receptor antagonists to the subject. One or more of the antagonists may be combined with a pharmaceutically acceptable carrier in a pharmaceutical composition (*e.g.*, pill, tablet, capsule). Furthermore, substances which antagonize multiple histamine receptors may be used in the present invention. For example, a subject can be administered one or more dual H3/H4 antagonists and, optionally, one or more H1 antagonists.

Also provided are combinations comprising one or more substances which antagonize the histamine H3 receptor, in association with one or more substances which antagonize histamine H4 receptor and, optionally, in association with one or more substances which antagonize histamine H1 receptor as well as pharmaceutical compositions, which comprise a pharmaceutically acceptable carrier, thereof. Pharmaceutical compositions are preferably in the form of a pill, capsule or tablet.

Preferred combinations comprise one or more H3 receptor antagonists, in association with one or more H4 receptor antagonists, or, alternatively, one or more dual H3/H4 receptor antagonists, in association with one or more H1 receptor antagonists. Another preferred combination comprises one or more dual H1/H3 antagonists in association with one or more H4 antagonists. Preferred antagonists are discussed, in detail, *infra*.

Preferably, one or more histamine H3 receptor antagonists are selected from thioperamide, impromidine, burimamide, clobenpropit, impentamine, mifetidine, clozapine, S-sopromidine, R-sopromidine, ciproxifam, SKF-91486 (3-(imidazole-4-yl)-propylguanidine sulfate), GR-175737 (Clitherow, *et al.*, (1996) Bioorg. Med. 6: 833–838), GT-2016 (Tedford, *et al.*, (1995) J. Pharm. Exp. Ther 275(2): 596-604), GT-2331 (Tedford, *et al.*, (1998) Eur. J. Pharmacol. 351(3): 307-11), GT-2394 (Yates, *et al.*, (2000) Soc. Neurosci. Abstr. 26: 279.), JB98064 (Linney, *et al.*, (2000) J. Med. Chem. 43: 2362–2370), UCL-1199 (Ganellin, *et al.*, (1995) J. Med. Chem. 38(17): 3342-50), ABT331440 (PCT Publication No. WO 02/06223),



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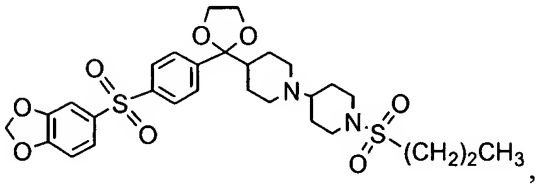

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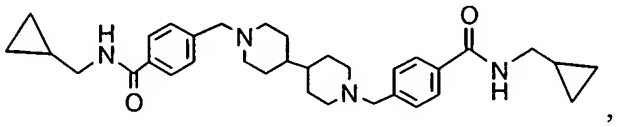

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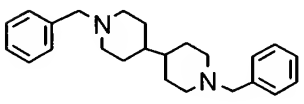

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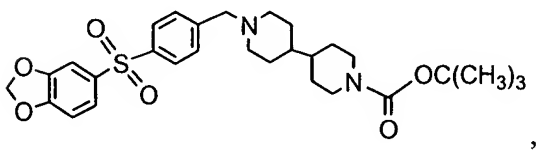

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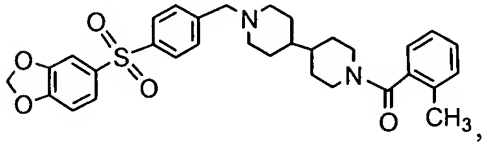

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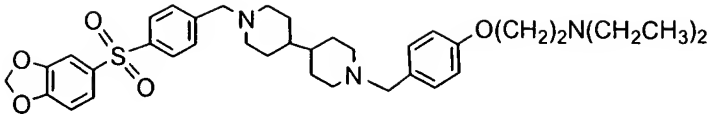
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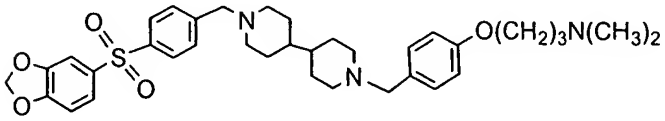
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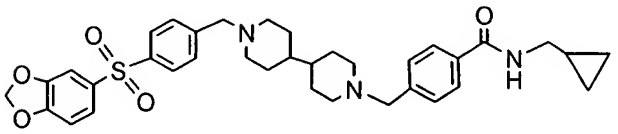
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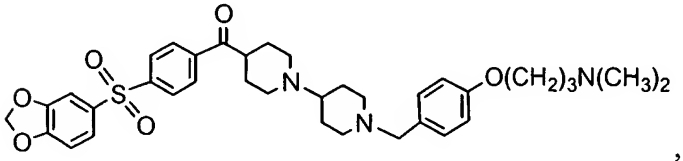
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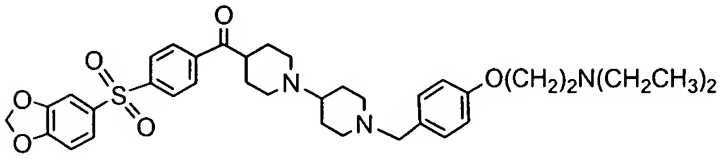
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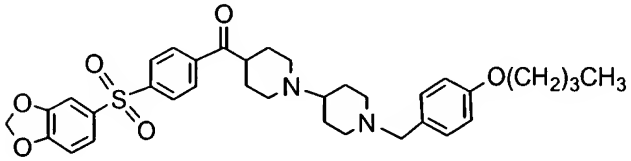
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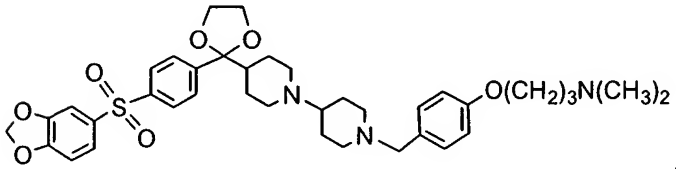
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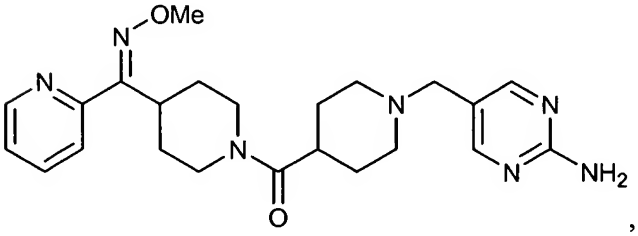
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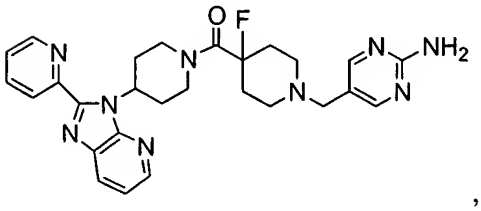
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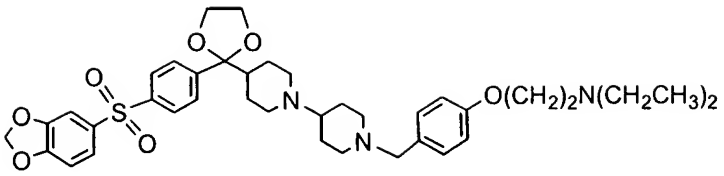

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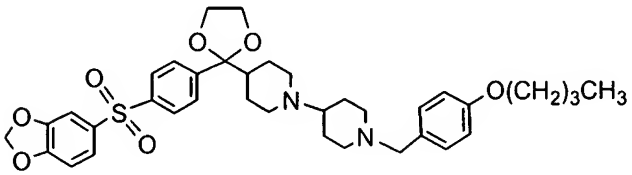
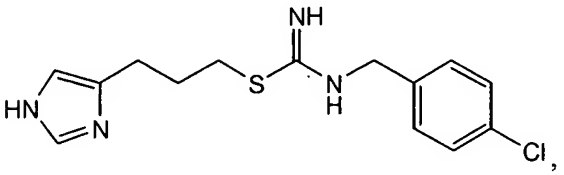


Preferably, one or more dual histamine H3 receptor/histamine H4 receptor antagonists are selected from



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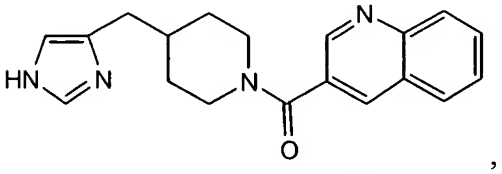

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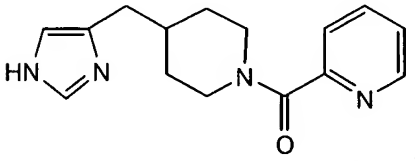

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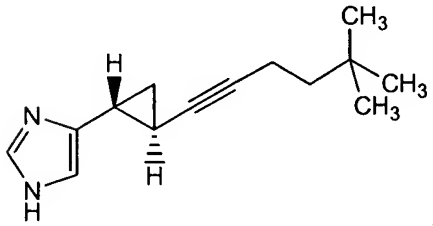

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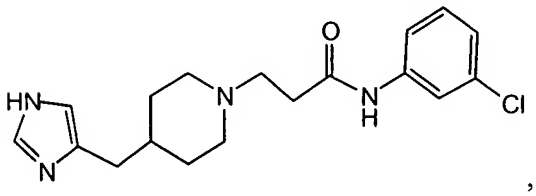

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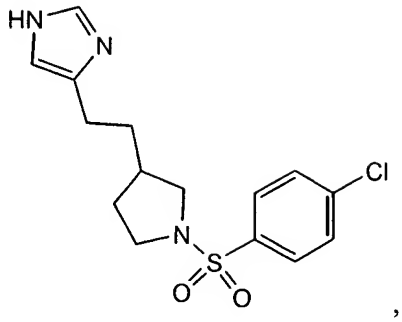
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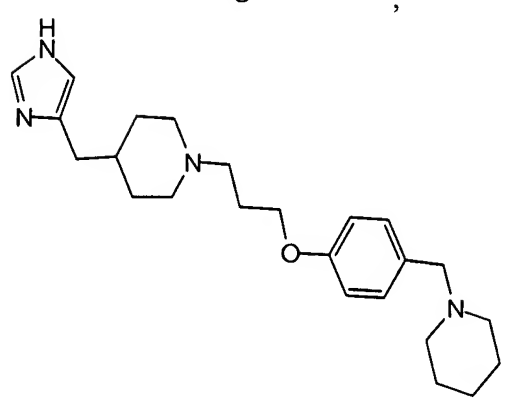
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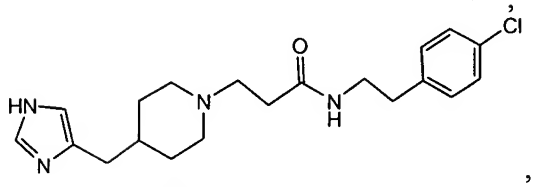
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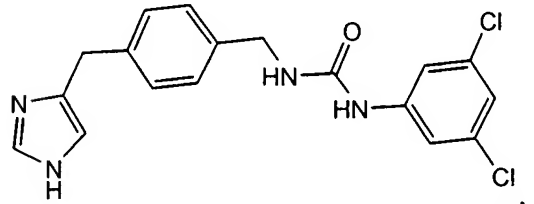
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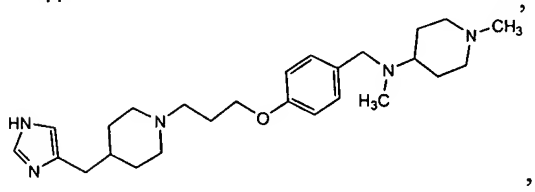
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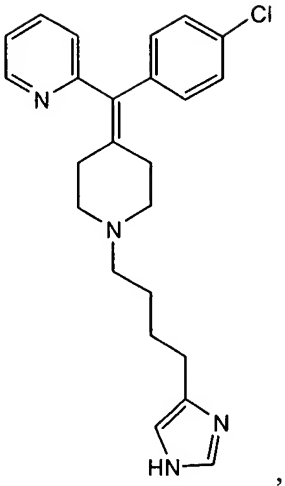
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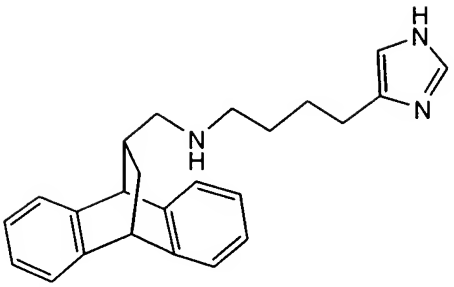
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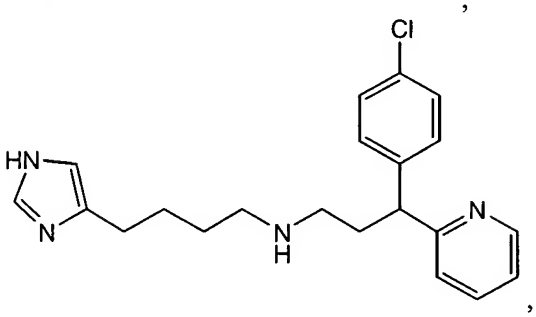
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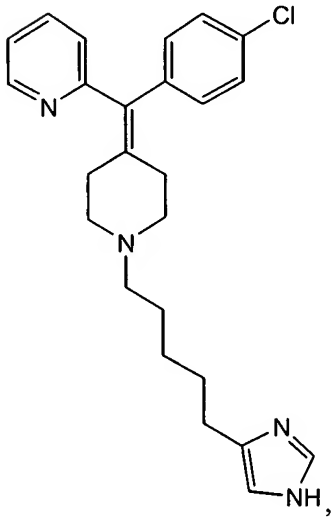
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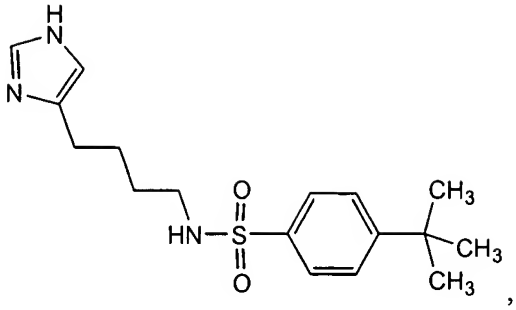

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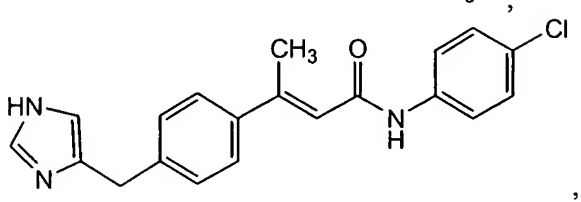

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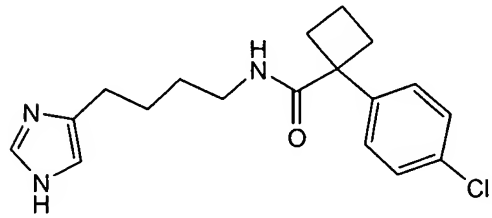

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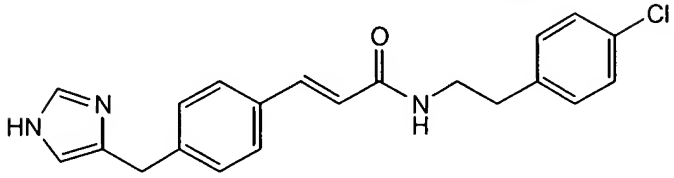

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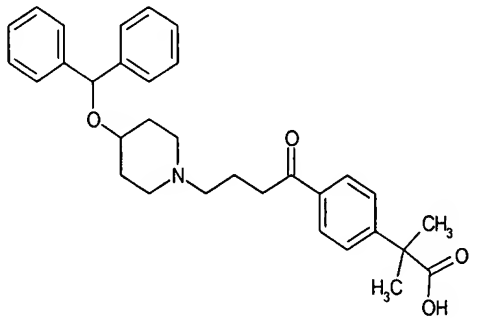
Preferably, one or more histamine H1 receptor antagonists are selected from astemizole, azatadine, azelastine, acrivastine, brompheniramine, cetirizine, chlorpheniramine, clemastine, cyclizine, carebastine, cyproheptadine, carbinoxamine, desloratadine, doxylamine, dimethindene, ebastine, epinastine, efletirizine, fexofenadine, hydroxyzine, ketotifen, loratadine, levocabastine, mizolastine, mequitazine, mianserin, noberastine, meclizine, norasternizole, picumast, pyrilamine, promethazine, terfenadine, tripelennamine, temelastine, trimeprazine, triprolidine and



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More preferably, one or more histamine H1 receptor antagonists are selected from loratadine, desloratadine, cetirizine and fexofenadine.

The antagonists of the present invention may be administered to the subject by any mode, such as parenterally or non-parenterally. Furthermore, the antagonists may be administered in a single composition.

DETAILED DESCRIPTION OF THE INVENTION

The present invention includes methods for administering one or more histamine receptor antagonists for the treatment or prevention of diseases and conditions which are mediated by the histamine receptors (*e.g.*, allergic rhinitis, congestion and pulmonary inflammation, preferably associated with asthma, chronic obstructive pulmonary disease (COPD), seasonal allergic rhinitis and perennial allergic rhinitis). Any antagonist or combination of antagonists which antagonize the histamine H3 receptor, the histamine H4 receptor and, optionally, the histamine H1 receptor may be administered to a subject for the purposes of the present invention. The antagonists can antagonize one or more histamine receptors. For example, a subject can be administered a single substance which antagonizes both a histamine H3 receptor and a histamine H4 receptor (dual H3/H4 antagonist) and optionally, an additional histamine H1 receptor antagonist.

The terms "H1" and "H1 receptor" both refer to a histamine H1 receptor. The terms "H3" and "H3 receptor" both refer to a histamine H3 receptor. The terms "H4" and "H4 receptor" both refer to a histamine H4 receptor.

The histamine H1 receptors, histamine H3 receptors and histamine H4 receptors of the invention may be from any organism, preferably a mammal (*e.g.*, horse, dog, cat, rat, mouse, rabbit, horse, pig and guinea pig) and most preferably a human. Genbank Accession No. AY136743 discloses a typical human histamine receptor H1, Genbank Accession No. AB045369 discloses a typical human histamine receptor H3 and Genbank Accession No. NM021624 discloses a typical human histamine receptor H4. Moreover, U.S. Patent No. 6,204,017 discloses a human histamine H4 receptor in SEQ ID NOs: 1 and 2 (SP9144).

The term "subject" includes any organism, preferably a mammal (*e.g.*, horse, dog, cat, rat, mouse, rabbit, horse, pig and guinea pig) and most preferably a human.

The term "in association with" indicates that the components of the combinations of the invention can be formulated into a single composition for simultaneous delivery or formulated separately into two or more compositions (*e.g.*, a kit). Furthermore, each component of a combination of the invention can be administered to a subject at a

different time than when the other component is administered; for example, each administration may be given non-simultaneously at several intervals over a given period of time. Moreover, the separate components may be administered to a subject by the same or by a different route (*e.g.*, orally, intranasally, intravenously).

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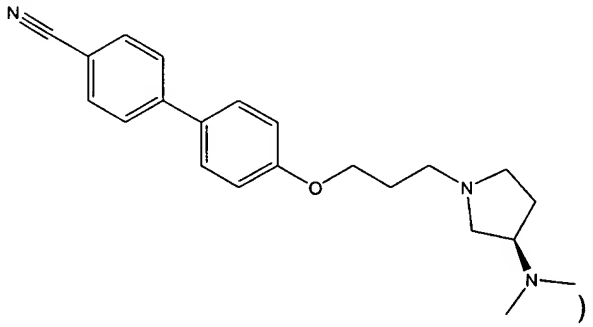
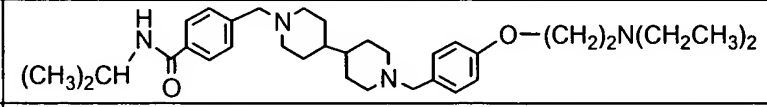
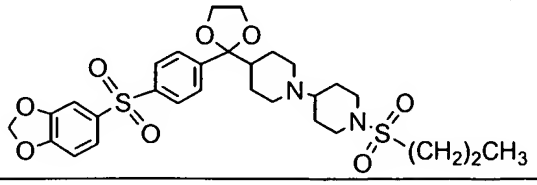
Histamine Receptor Antagonists

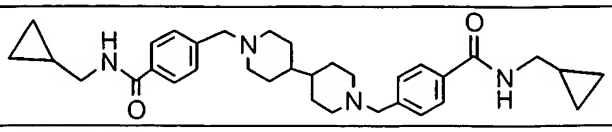
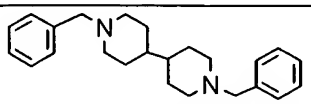
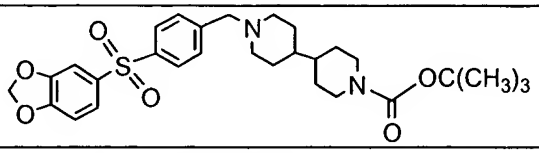
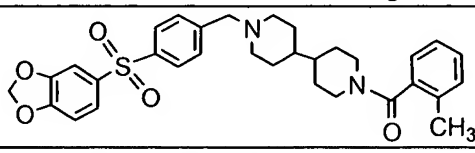
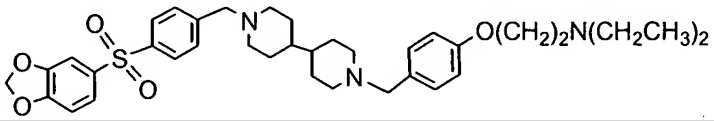
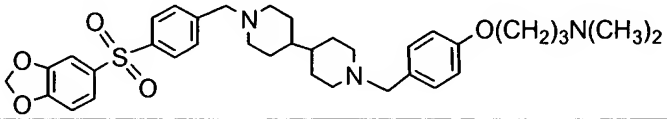
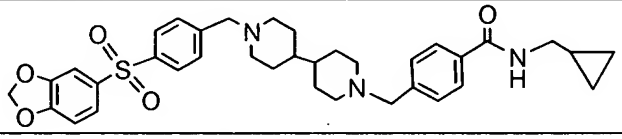
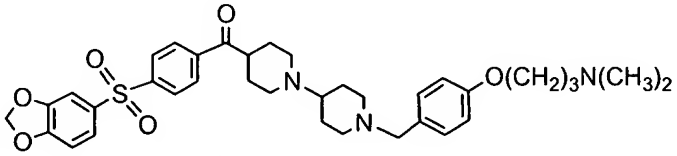
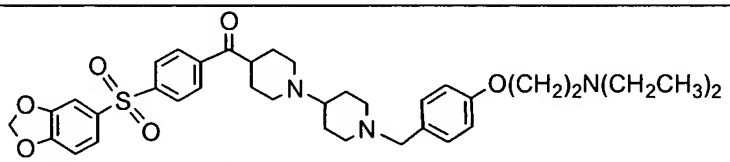
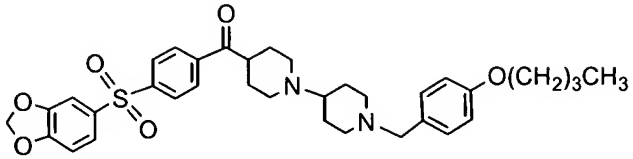
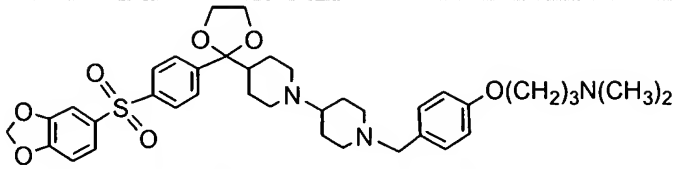
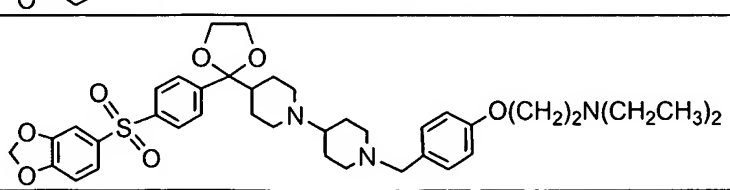
Histamine H3 receptor antagonists include, without limitation: thioperamide, impromidine, burimamide, clobenpropit, impentamine, mifetidine, clozapine, S-sopromidine, R-sopromidine, ciproxifam, SKF-91486 (3-(imidazole-4-yl)-propylguanidine sulfate), GR-175737 (Clitherow, *et al.*, (1996) Bioorg. Med. 6: 833–838), GT-2016 (Tedford, *et al.*, (1995) J. Pharm. Exp. Ther 275(2): 596-604), GT-2331 (Tedford, *et al.*, (1998) Eur. J. Pharmacol. 351(3): 307-11), GT-2394 (Yates, *et al.*, (2000) Soc. Neurosci. Abstr. 26: 279.), JB98064 (Linney, *et al.*, (2000) J. Med. Chem. 43: 2362–2370), UCL-1199 (Ganellin, *et al.*, (1995) J. Med. Chem. 38(17): 3342-50) and ABT331440 (PCT Publication No. WO 02/06223;

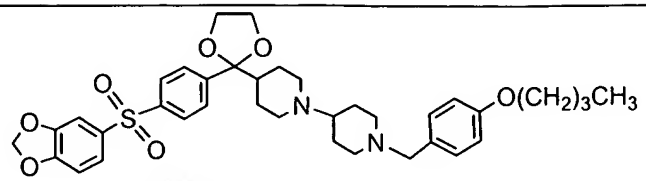
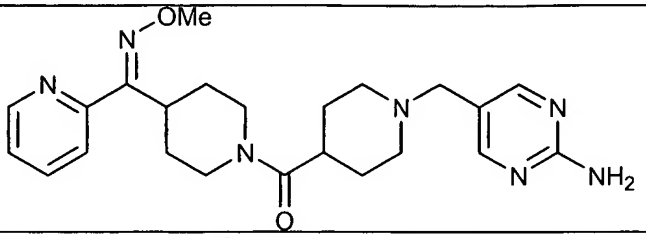
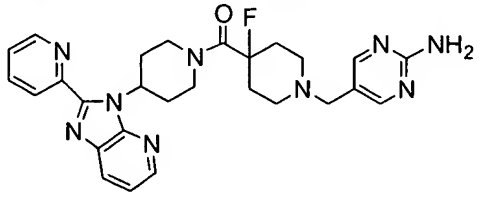


Other exemplary histamine H3 receptor antagonists are set forth, below, in Table 1.

Table 1. Histamine H3 Receptor Antagonists.

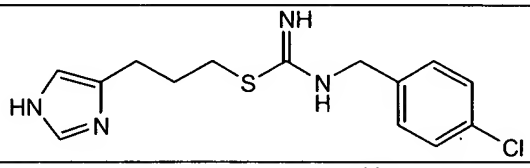
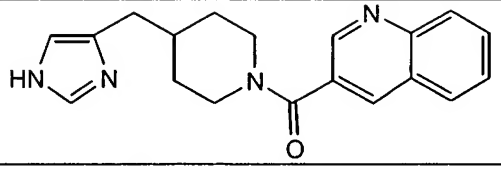
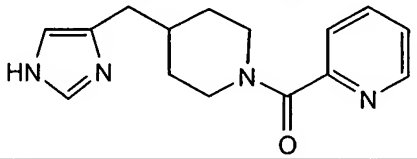
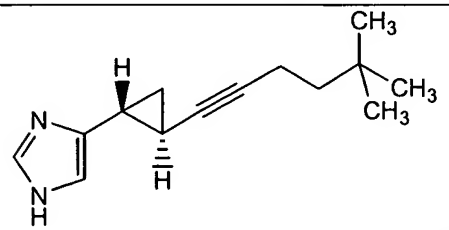
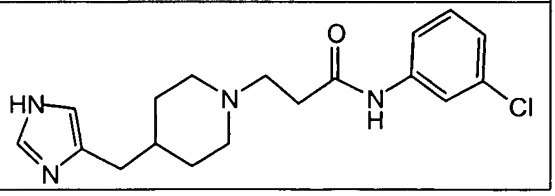
Formula	Structure
1	
2	

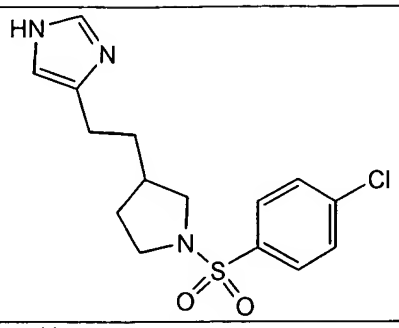
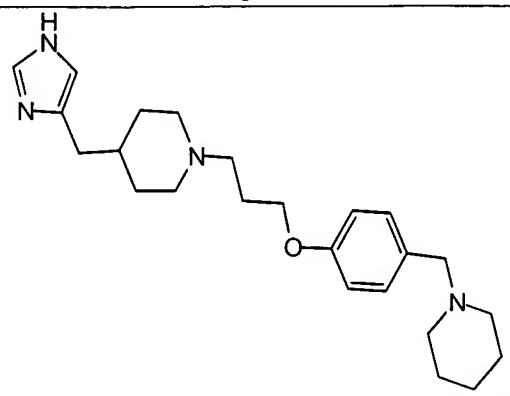
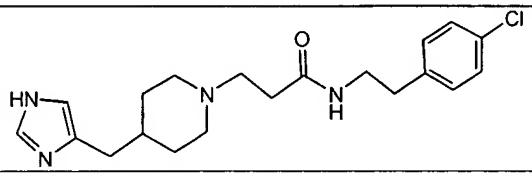
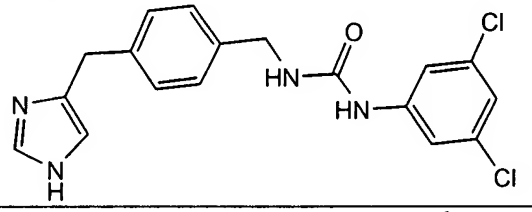
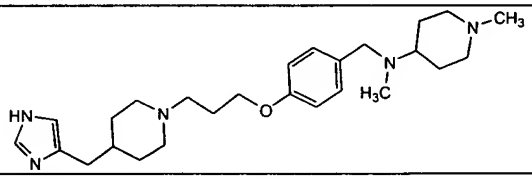
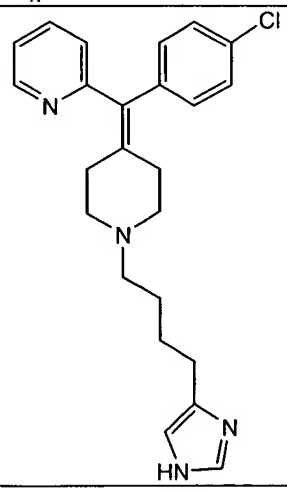
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	

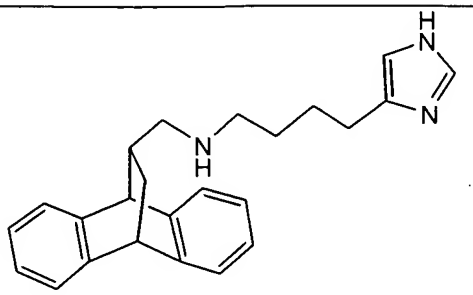
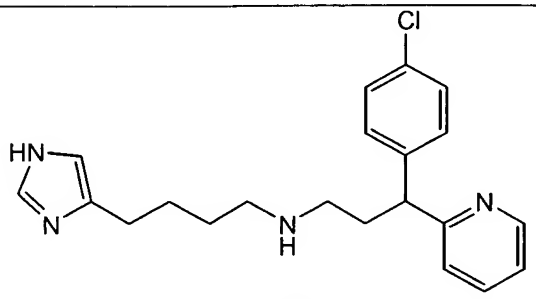
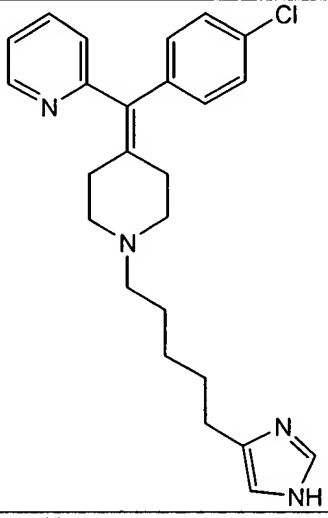
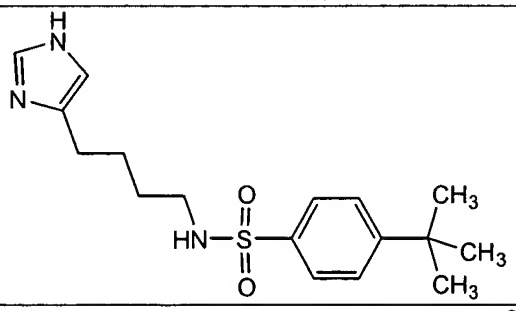
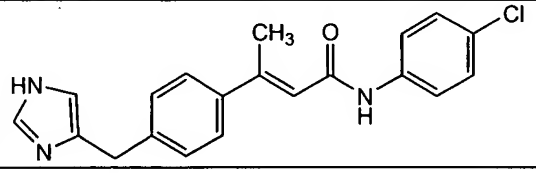
15	
16	
17	

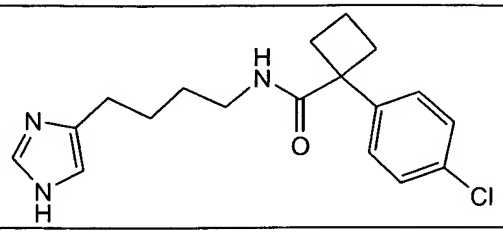
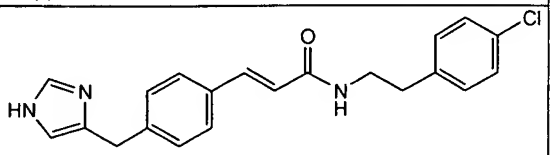
Exemplary, dual H3/H4 receptor antagonists are shown, below, in Table 2.

Table 2. Dual H3/H4 Receptor Antagonists.

Formula	Structure
18	
19	
20	
21	
22	

23	
24	
25	
26	
27	
28	

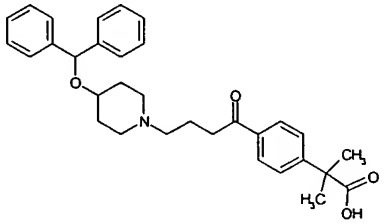
29	
30	
31	
32	
33	

34	
35	

Preferably, the dual H3/H4 receptor antagonist is selected from compounds comprising a formula selected from formulas 18, 19, 20, 20, 22, 23, 24, 26, 28, 31, 32, 33 and 35.

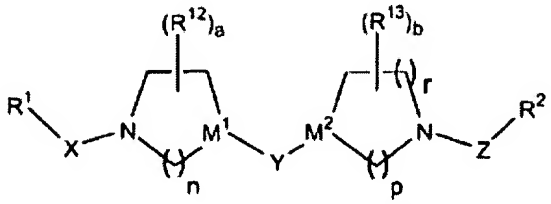
A H4 receptor antagonist can also be any one or more of those disclosed in Jablonowski *et al.*, J. Med. Chem. 46:3957-3960 (2003), particularly compound 6, and/or compound 10e and/or compound 101 therein.

Numerous chemical substances are known to have histamine H1 receptor antagonist activity. Many useful compounds can be classified as ethanolamines, ethylenediamines, alkylamines, phenothiazines or piperidines. Representative H1 receptor antagonists include, without limitation: astemizole, cetirizine, azatadine, azelastine, acrivastine, brompheniramine, chlorpheniramine, clemastine, cyclizine, carebastine, cyproheptadine, carbinoxamine, desloratadine, doxylamine, dimethindene, ebastine, epinastine, efletirizine, fexofenadine, hydroxyzine, ketotifen, loratadine, levocabastine, mizolastine, mequitazine, mianserin, noberastine, meclizine, norasternizole, picumast, pyrilamine, promethazine, terfenadine, tripelennamine, temelastine, trimeprazine, triprolidine and the compound of formula 36:



Histamine H3 receptor antagonists which are part of the present invention are disclosed in several U.S. patents, applications and publications:

PCT Publication No. WO 02/72570 discloses compounds comprising the following structural formula:



(I)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

(A) R^1 is selected from:

(1) aryl;

(2) heteroaryl;

(3) heterocycloalkyl

(4) alkyl;

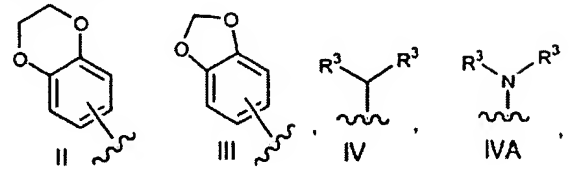
(5) $-C(O)N(R^{4B})_2$;

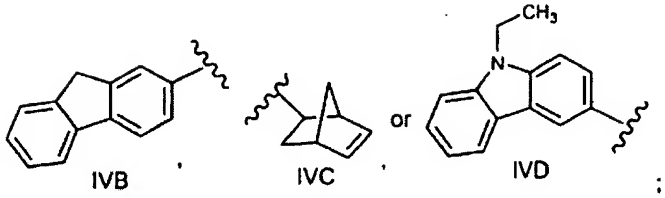
(6) cycloalkyl;

(7) arylalkyl;

(8) heteroarylheteroaryl (e.g., isoxazoylthienyl or pyridylthienyl); or

(9) a group selected from:





said aryl (see (A)(1) above), heteroaryl (see (A)(2) above), aryl portion of arylalkyl (see (A)(7) above), phenyl ring of formula II (see (A)(9) above), phenyl ring of formula III (see (A)(9) above), phenyl rings of formula IVB (see (A)(9) above), or phenyl rings of formula IVD (see (A)(9) above) are optionally substituted with 1 to 3 substituents independently selected from:

(1) halogen (e.g., Br, F, or Cl, preferably F or Cl);

(2) hydroxyl (i.e., -OH);

(3) lower alkoxy (e.g., C_1 to C_6 alkoxy, preferably C_1 to C_4 alkoxy, more preferably C_1 to C_2 alkoxy, most preferably methoxy);

(4) -Oaryl (i.e., aryloxy);

(5) $-SR^{22}$;

(6) $-CF_3$;

(7) $-OCF_3$;

(8) $-OCHF_2$;

(9) $-NR^4R^5$;

(10) phenyl;

(11) NO_2,

(12) $-CO_2R^4$;

(13) $-CON(R^4)_2$ wherein each R^4 is the same or different;

(14) $-S(O)_2R^{22}$;

(15) $-S(O)_2N(R^{20})_2$ wherein each R^{20} is the same or different;

(16) $-N(R^{24})S(O)_2R^{22}$;

(17) -CN;

(18) $-CH_2OH$;

(19) $-OCH_2CH_2OR^{22}$;

(20) alkyl (e.g., C_1 to C_4, such as methyl);

(21) substituted phenyl wherein said phenyl has 1 to 3 substituents independently selected from alkyl, halogen, -CN, $-NO_2$, $-OCHF_2$, -Oalkyl;

(22) -Oalkylaryl (preferably –Oalkylphenyl or –Oalkyl-substituted phenyl, e.g., $-OCH_2$dichlorophenyl, such as $-OCH_2$-2,6-dichlorophenyl or $-OCH_2$-2-chloro-6-fluorophenyl) wherein said aryl group is optionally substituted with 1 to 3 independently selected halogens; or

(23) phenyl;

(B) X is selected from alkyl (e.g., $-(CH_2)_q-$ or branched alkyl) or $-S(O)_2-$;

(C) Y represents

(1) a single bond (i.e., Y represents a direct bond from M^1 to M^2); or

(2) Y is selected from –C(O)-, -C(S)-, $-(CH_2)_q-$, or $-NR^4C(O)-$; with the provisos that:

(a) when M^1 is N, then Y is not $-NR^4C(O)-$; and

(b) when Y is a bond, then M^1 and M^2 are both carbon;

(D) M^1 and M^2 are independently selected from C or N;

(E) Z is selected from: C_1-C_6 alkyl, $-SO_2-$, -C(O)- or $-C(O)NR^4-$;

(F) R^2 is selected from:

(1) a six-membered heteroaryl ring having 1 or 2 heteroatoms independently selected from N or N-O (i.e., N-oxide), with the remaining ring atoms being carbon;

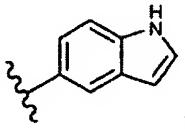
(2) a five-membered heteroaryl ring having 1 to 3 heteroatoms selected from nitrogen, oxygen, or sulfur with the remaining ring atoms being carbon; or

(3) an alkyl group, preferably a C_1 to C_4 alkyl group, more preferably methyl;

(4) an aryl group, e.g., phenyl or substituted phenyl (preferably phenyl), wherein said substituted phenyl is substituted with 1 to 3 substituents independently selected from: halogen, -Oalkyl, $-OCF_3$, $-CF_3$, -CN, $-NO_2$, $-NHC(O)CH_3$, or $-O(CH_2)_qN(R^{10A})_2$;

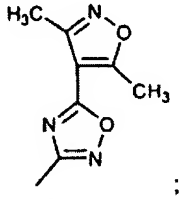
(5) $-N(R^{11A})_2$ wherein each R^{11A} is independently selected from: H, alkyl (e.g., i-propyl) or aryl (e.g., phenyl), preferably one R^{11A} is H and the other is phenyl or alkyl (e.g., i-propyl);

(6) a group of the formula:



; or

(7) a heteroarylheteroaryl group, e.g.,



;

said five membered heteroaryl ring ((F)(2) above) or six-membered heteroaryl ring ((F)(1) above) is optionally substituted with 1 to 3 substituents selected from:

(a) halogen;

(b) hydroxyl;

(c) lower alkyl;

(d) lower alkoxy;

(e) $-CF_3$;

(f) $-NR^4R^5$;

(g) phenyl;

(h) $-NO_2$;

(i) $-C(O)N(R^4)_2$ (wherein each R^4 is the same or different);

(j) $-C(O)_2R^4$; or

(k) phenyl substituted with 1 to 3 substituents independently selected from: halogen, -Oalkyl, $-OCF_3$, $-CF_3$, -CN, $-NO_2$ or $-O(CH_2)_qN(R^{10A})_2$;

(G) R^3 is is selected from:

(1) aryl;

(2) heteroaryl;

(3) heterocycloalkyl

(4) alkyl; or

(5) cycloalkyl;

wherein said aryl or heteroaryl R^3 groups is optionally substituted with 1 to 3 substituents independently selected from:

(a) halogen (e.g., Br, F, or Cl, preferably F or Cl);

(b) hydroxyl (i.e., -OH);

(c) lower alkoxy (e.g., C_1 to C_6 alkoxy, preferably C_1 to C_4 alkoxy, more preferably C_1 to C_2 alkoxy, most preferably methoxy);

(d) -Oaryl (i.e., aryloxy);

(e) $-SR^{22}$;

(f) $-CF_3$;

(g) $-OCF_3$;

(h) $-OCHF_2$;

(i) $-NR^4R^5$;

(j) phenyl;

(k) $-NO_2$,

(l) $-CO_2R^4$;

(m) $-CON(R^4)_2$ wherein each R^4 is the same or different;

(n) $-S(O)_2R^{22}$;

(o) $-S(O)_2N(R^{20})_2$ wherein each R^{20} is the same or different;

(p) $-N(R^{24})S(O)_2R^{22}$;

(q) -CN;

(r) $-CH_2OH$;

(s) $-OCH_2CH_2OR^{22}$; or

(t) alkyl;

(H) R^4 is selected from:

(1) hydrogen;

(2) C_1-C_6 alkyl;

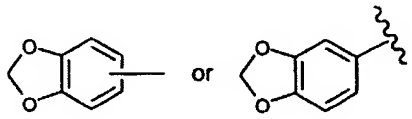
(3) cycloalkyl;

(4) cycloalkylalkyl (e.g., cyclopropyl-CH_2- or cyclohexyl-CH_2-);

(5) heterocycloalkylalky (e.g., tetrahydrofuranyl-CH_2-);

(6) bridged bicyclic cycloalkyl ring, such as, for example:

(7) aryl having a fused heterocycloalkyl ring bound to said aryl ring, preferably the heteroatoms in said heterocycloalkyl ring are two oxygen atoms, e.g., phenyl having a heterocycloalkyl ring bound to said phenyl ring, such as

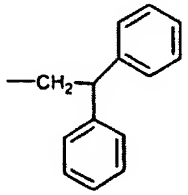


(8) aryl;

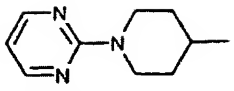
(9) arylalkyl;

(10) alkylaryl;

(11) -$(CH_2)_dCH(R^{12A})_2$ wherein d is 1 to 3 (preferably 1), and each R^{12A} is independently selected from phenyl or substituted phenyl, said substituted phenyl being substituted with 1 to 3 substituents independently selected from: halogen, -Oalkyl, -OCF_3, -CF_3, -CN, or -NO_2, e.g.,



(12) heterocycloalkylheteroaryl, e.g.,

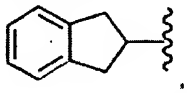

; or

(13) -(C_1 to C_6)alkylene-O-R^{22} (e.g., -$C_3H_6OCH_3$);

wherein the aryl R^4 group, the aryl portion of the arylalkyl R^4 group, or the aryl portion of the alkylaryl R^4 group is optionally substituted with 1 to 3 substituents independently selected from:

(a) halogen;
(b) hydroxyl;
(c) lower alkyl;
(d) lower alkoxy;
(e) -CF_3;
(f) -$N(R^{20})(R^{24})$,
(g) phenyl;
(h) -NO_2;
(i) –$C(O)N(R^{20})_2$ (wherein each R^{20} is the same or different),
(j) -$C(O)R^{22}$;
(i) -$(CH_2)_k$-cycloalkyl;
(j) -$(CH_2)_q$-aryl; or
(k) -$(CH_2)_m$-OR^{22};

(l) each R^{4B} is independently selected from: H, heteroaryl (e.g., pyridyl), alkyl, alkenyl (e.g., allyl), a group of the formula



arylalkyl (e.g., benzyl), or arylalkyl wherein the aryl moiety is substitued with 1-3 substituents independently selected from: halogen (e.g. –CH_2-p-Clphenyl); preferably one R^{4B} is H;

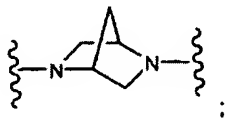
(J) R^5 is selected from: hydrogen, C_1-C_6 alkyl, -$C(O)R^{20}$ (e.g., -C(O)alkyl, such as –$C(O)CH_3$), -$C(O)_2R^{20}$, –$C(O)N(R^{20})_2$ (wherein each R^{20} is the same or different);

(K) each R^{10A} is independently selected from H or C_1 to C_6 alkyl (e.g., methyl), or each R^{10A}, taken together with the nitrogen atom to which they are bound, forms a 4 to 7 membered heterocycloalkyl ring;

(L) R^{12} is

(1) selected from alkyl, hydroxyl, alkoxy, or fluoro, provided that when R^{12} is hydroxy or fluoro then R^{12} is not bound to a carbon adjacent to a nitrogen; or

(2) R^{12} forms an alkyl bridge from one ring carbon to another ring carbon, an example of such a bridged ring system is:

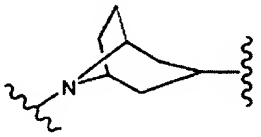


;

(M) R^{13} is

(1) selected from alkyl, hydroxyl, alkoxy, or fluoro, provided that when R^{13} is hydroxy or fluoro then R^{13} is not bound to a carbon adjacent to a nitrogen; or

(2) R^{13} forms an alkyl bridge from one ring carbon to another ring carbon, an example of such a bridged ring system is:



(N) R^{20} is selected from hydrogen, alkyl, or aryl, wherein said aryl group is optionally substituted with from 1 to 3 groups independently selected from: halogen, $-CF_3$, $-OCF_3$, hydroxyl, or methoxy; or when two R^{20} groups are present, said two R^{20} groups taken together with the nitrogen to which they are bound form a five or six membered heterocyclic ring;

(O) R^{22} is selected from: heterocycloalkyl (e.g., morpholinyl or pyrrolidinyl), alkyl or aryl, wherein said aryl group is optionally substituted with 1 to 3 groups independently selected from halogen, $-CF_3$, $-OCF_3$, hydroxyl, or methoxy;

(P) R^{24} is selected from: hydrogen, alkyl, $-SO_2R^{22}$, or aryl, wherein said aryl group is optionally substituted with 1 to 3 groups independently selected from halogen, $-CF_3$, $-OCF_3$, hydroxyl, or methoxy;

(Q) a is 0 to 2;

(R) b is 0 to 2;

(S) k is 1 to 5;

(T) m is 2 to 5;

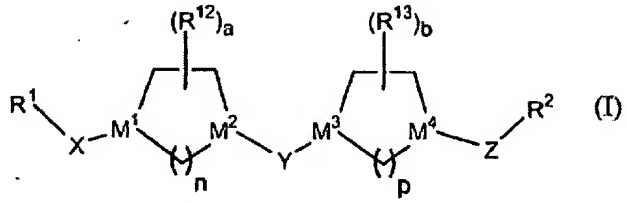
(U) n is 1, 2 or 3 with the proviso that when M^1 is N, then n is not 1;

(V) p is 1, 2 or 3 with the proviso that when M^2 is N, then p is not 1;

(W) q is 1 to 5; and

(X) r is 1, 2, or 3 with the proviso that when r is 2 or 3, then M^2 is C and p is 1.

PCT Publication No WO 02/32893 discloses compounds comprising the following structural formula:



(I)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

(1) R^1 is is selected from:

(a) aryl;

(b) heteroaryl;

(c) heterocycloalkyl

(d) alkyl;

(e) cycloalkyl; or

(f) alkylaryl;

wherein said R^1 groups are optionally substituted with 1 to 4 substituents independently selected from:

(1) halogen (e.g., Br, F, or Cl, preferably F or Cl);

(2) hydroxyl (i.e., -OH);

(3) lower alkoxy (e.g., C_1 to C_6 alkoxy, preferably C_1 to C_4 alkoxy, most preferably C_1 to C_2 alkoxy, more preferably methoxy);

(4) $-CF_3$;

(5) CF_3O-;

(6) $-NR^4R^5$;

(7) phenyl;

(8) $-NO_2$,

(9) $-CO_2R^4$;

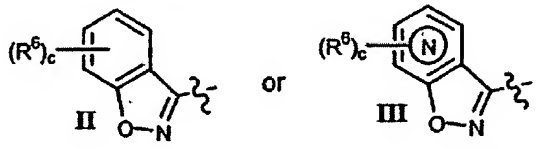
(10) $-CON(R^4)_2$ wherein each R^4 is the same or different;

(11) $-S(O)_mN(R^{20})_2$ wherein each R^{20} is the same or different H or alkyl group, preferably C_1 to C_4 alkyl, most preferably C_1-C_2 alkyl, and more preferably methyl;

(12) -CN; or

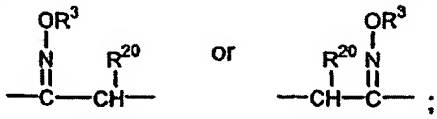
(13) alkyl; or

(2) R^1 and X taken together form a group selected from:



;

(3) X is selected from: =C(O), =C(NOR^3), =C(NNR^{4}R^5),



;

(4) M^1 is carbon;

(5) M^2 is selected from C or N;

(6) M^3 and M^4 are independently selected from C or N;

(7) Y is selected from: is $-CH_2-$, =C(O), $=C(NOR^{20})$ (wherein R^{20} is as defined above), or =C(S);

(8) Z is a $C_1 - C_6$ alkyl group;

(9) R^2 is a five or six-membered heteroaryl ring, said six-membered heteroaryl ring comprising 1 or 2 nitrogen atoms with the remaining ring atoms being carbon, and said five-membered heteroaryl ring containing 1 or 2 heteroatoms selected from: nitrogen, oxygen, or sulfur with the remaining ring atoms being carbon; said five or six membered heteroaryl rings being optionally substituted with 1 to 3 substituents independently selected from: halogen, hydroxyl, lower alkyl, lower alkoxy, $-CF_3$, CF_3O-, $-NR^4R^5$, phenyl, $-NO_2$, $-CO_2R^4$, $-CON(R^4)_2$ wherein each R^4 is the same or different, $-CH_2NR^4R^5$, $-(N)C(NR^4R^5)_2$, or -CN;

(10) R^3 is selected from:

(a) hydrogen;

(b) $C_1 - C_6$ alkyl;

(c) aryl;

(d) heteroaryl;

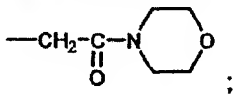
(e) heterocycloalkyl;

(f) arylalkyl (e.g., aryl(C_1 to C_4)alkyl, e.g., $-(CH_2)_w$aryl wherein w is 1 to 4, preferably 1 or 2, and most preferably 1, such as, for example $-CH_2$phenyl or $-CH_2$substituted phenyl);

(g) $-(CH_2)_e-C(O)N(R^4)_2$ wherein each R^4 is the same or different,

(h) $-(CH_2)_e-C(O)OR^4$;

(i) $-(CH_2)_e-C(O)R^{30}$ wherein R^{30} is a heterocycloalkyl group, such as, for example, morpholinyl, piperidinyl, piperazinyl or pyrrolidinyl, including



;

(j) $-CF_3$; or

(k) $-CH_2CF_3$;

wherein said aryl, heteroaryl, heterocycloalkyl, and the aryl portion of said arylalkyl are optionally substituted with 1 to 3 (preferably 1) substituents selected from: halogen (e.g., F or Cl), -OH, $-OCF_3$, $-CF_3$, -CN, $-N(R^{45})_2$, $-CO_2R^{45}$, or $-C(O)N(R^{45})_2$, wherein each R^{45} is independently selected from: H, alkyl, alkylaryl, or alkylaryl wherein said aryl moiety is substituted with 1 to 3 substituents independently selected from $-CF_3$, -OH, halogen, alkyl, $-NO_2$, or -CN;

(11) R^4 is selected from: hydrogen, $C_1 - C_6$ alkyl, aryl, alkylaryl, said aryl and alkylaryl groups being optionally substituted with 1 to 3 substituents selected from: halogen, $-CF_3$, $-OCF_3$, -OH, $-N(R^{45})_2$, $-CO_2R^{45}$, $-C(O)N(R^{45})_2$, or –CN; wherein R^{45} is as defined above;

(12) R^5 is selected from: hydrogen, $C_1 - C_6$ alkyl, $-C(O)R^4$, $-C(O)_2R^4$, or $-C(O)N(R^4)_2$ wherein each R^4 is independently selected, and R^4 is as defined above;

(13) or R^4 and R^5 taken together with the nitrogen atom to which they are bound forms a five or six membered heterocycloalkyl ring (e.g., morpholine);

(14) R^6 is selected from: alkyl, aryl, alkylaryl, halogen, hydroxyl, lower alkoxy, $-CF_3$, CF_3O-, $-NR^4R^5$, phenyl, $-NO_2$, $-CO_2R^4$, $-CON(R^4)_2$ wherein each R^4 is the same or different, or -CN;

(15) R^{12} is selected from: alkyl, hydroxyl, alkoxy, or fluoro;

(16) R^{13} is selected from: alkyl, hydroxyl, alkoxy, or fluoro;

(17) a (subscript for R^{12}) is 0 to 2;

(18) b (subscript for R^{13}) is 0 to 2;

(19) c (subscript for R^6) is 0 to 2;

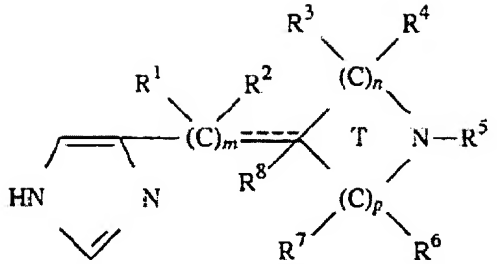
(20) e is 0 to 5;

(21) m is 1 or 2;

(22) n is 1, 2 or 3; and

(23) p is 1, 2 or 3, with the proviso that when M^3 and M^4 are both nitrogen, then p is 2 or 3 (i.e., p is not 1 when M^3 and M^2 are both nitrogen).

U.S. Patent No. 5,463,074 discloses compounds comprising the following structural formula:



(I)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

(A) m is an integer selected from the group consisting of: 0, 1, and 2;

(B) n and p are integers and are each independently selected from the group consisting of: 0, 1, 2, and 3 such that the sum of n and p is 2 or 3 such that when the sum of n and p is 2, T is a 4-membered ring and when the sum of n and p is 3, T is a 5-membered ring;

(C) each R^1, R^2, R^3, R^4, R^6, R^7, and R^8 is independently selected from the group consisting of:

(1) H;

(2) C_1 to C_6 alkyl;

(3) C_3 to C_6 cycloalkyl; and

(4) $-(CH_2)_q-R^9$ wherein q is an integer of: 1 to 7, and R^9 is selected from the group consisting of: phenyl, substituted phenyl, $-OR^{10}$, $-C(O)OR^{10}$, $-C(O)R^{10}$, $-OC(O)R^{10}$, $-C(O)NR^{10}R^{11}$, CN and $-SR^{10}$ wherein R^{10} and R^{11} are as defined below,

and wherein the substituents on said substituted phenyl are each independently selected from the group consisting of: —OH, —O—(C_1 to C_6)alkyl, halogen, C_1 to C_6 alkyl, —CF_3, —CN, and —NO_2, and wherein said substituted phenyl contains from 1 to 3 substituents; examples of —$(CH_2)_1$—R^9 include benzyl, substituted benzyl and the like, wherein the substituents on the substituted benzyl are as defined above for said substituted phenyl;

(D) R^5 is selected from the group consisting of:

(1) H;

(2) C_1 to C_{20} alkyl;

(3) C_3 to C_6 cycloalkyl;

(4) —$C(O)OR^{10'}$; wherein $R^{10'}$ is the same as R^{10} defined below except that $R^{10'}$ is not H;

(5) —$C(O)R^{10}$;

(6) —$C(O)NR^{10}R^{11}$;

(7) allyl;

(8) propargyl; and

(9) —$(CH_2)_q$—R^9, wherein q and R^9 are as defined above with the proviso that when q is 1 when R^9 is not —OH or —SH;

(E) R^{10} and R^{11} are each independently selected from the group consisting of: H, C_1 to C_6 alkyl, and C_3 to C_6 cycloalkyl; and, for the substituent —$C(O)NR^{10}R^{11}$, R^{10} and R^{11}, together with the nitrogen to which they are bound, can form a ring having 5, 6, or 7 atoms;

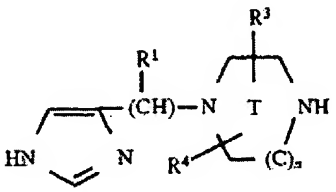
(F) the dotted line (. . .) represents a double bond that is optionally present when m is 1, and T is a 5-membered ring, and n is not 0, and p is not 0(i.e., the nitrogen in the ring is not bound directly to the carbon atom bearing the double bond), and when said double bond is present then R^2 and R^8 are absent;

(G) when m is 2, each R^1 is the same or different substituent for each m, and each R^2 is the same or different substituent for each m;

(H) when n is 2 or 3, each R^3 is the same or different substituent for each n, and each R^4 is the same or different substituent for each n, and

(I) when p is 2 or 3, each R^6 is the same or different substituent for each p, and each R^7 is the same or different substituent for each p.

U.S. Patent No. 5,633,250 discloses compounds comprising the following structural formula:



(1.0)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

(A) n is 1 or 2, such that when n is 1 then ring T is a six membered ring, and when n is 2 then ring T is a seven membered ring;

(B) R^1 is selected from the group consisting of:
(1) H;
(2) C_1 to C_6 alkyl;
(3) allyl; and
(4) propargyl;

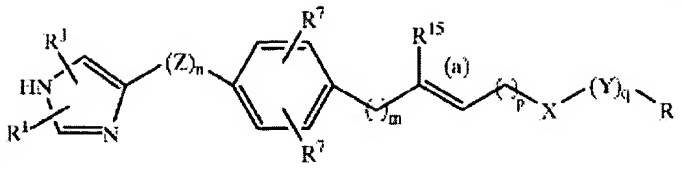
(C) R^3 and R^4 are independently selected from the group consisting of:
(1) H;
(2) C_1 to C_6 alkyl;
(3) allyl;
(4) propargyl; and
(5) $-(CH_2)_q-R^5$ wherein q is an integer of: 1 to 7, and R^5 is selected from the group consisting of: phenyl, substituted phenyl, $-OR^6$, $-C(O)OR^6$, $-C(O)R^6$, $-OC(O)R^6$, $-C(O)NR^6R^7$, CN and $-SR^6$ wherein R^6 and R^7 are as defined below, and wherein the substituents on said substituted phenyl are each independently selected from the group consisting of: —OH, —O—(C_1 to C_6)alkyl, halogen, C_1 to C_6 alkyl, $-CF_3$, —CN, and $-NO_2$, and wherein said substituted phenyl contains from 1 to 3 substituents;

(D) R^6 and R^7 are each independently selected from the group consisting of: H and C_1 to C_6 alkyl; and

(E) R^3 and R^4 are each independently bound to the same or different carbon atom of ring T.

U.S. Patent No. 6,034,251 discloses compounds comprising the following structural formula:

(I)



or a pharmaceutically acceptable salt or solvate thereof, wherein:

the double bond (a) is E or Z (that is the double bond to the carbon atom having the R^{15} substituent is of the E or Z configuration);

each R^1 is independently selected from the group consisting of hydrogen, lower alkyl, trihalomethyl, phenyl and benzyl;

each R^7 is independently selected from the group consisting of hydrogen, lower alkyl, halogen, trihalomethyl, $NR^{10}R^{11}$, or a group OR^{10}, whereby R^{10} and R^{11} are independently selected from hydrogen, lower alkyl or trihalomethyl;

X is $-CONR^5-$; $-SO_2-$, $-S-$; $-CO-$; $-COO-$; $-CN(OR^5)NR^5-$; $-C(NR^5)NR^5-$; $-SONR^5-$; $-SO_2NR^5-$ and, provided p is not zero, X may also be $-O-$; $-NR^5-$; $-NR^5CONR^5-$; $-OCONR^5-$; $-O-CO-$ or $-NR^5CO-$;

Y is C_1–C_3-alkyl, optionally substituted at any carbon atom of the group by one substituent R^5;

Z is $C(R^1)_2$; wherein no more than two R^1 groups are other than hydrogen;

n is 1 or 2;

m is 0 or 1;

p is 0 or 1;

q is 0 or 1;

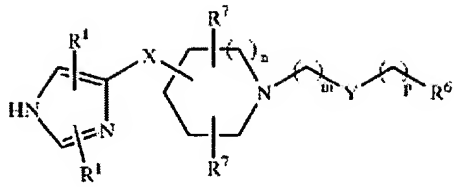
R is selected from C_3 to C_7 cycloalkyl, heterocyclic groups, aryl or heteroaryl, wherein said R groups are optionally substituted with 1–3 substituents as defined below;

each R^5 independently represents hydrogen, lower alkyl or poly-halologweralkyl; and

R^{15} represents H or lower alkyl (e.g., methyl).

U.S. Patent No. 6,100,279 discloses compounds comprising the following structural formula:

(I)



or pharmaceutically acceptable salts or solvates thereof, wherein:

X is a straight chain alkyl group having 1 to 7 carbon atoms or an alkene or alkyne group with 2 to 4 carbon atoms; wherein said alkyl or alkene groups are optionally substituted with up to two (i.e., 1 or 2) R^7 groups;

n is 0,1 or 2,

m and p are 0 to 4;

when m is 0 to 4, Y represents —SO_2—; —CS—; —CO—; —$CONR^5$—; —$CO(CH_2)_wO$— (with w being 1 to 4); —COO—; —$CON(OR^5)$—; —$C(NR^5)NR^5$—; —SO_2NR^5— or —$CSNR^5$—;

when m is 2 to 4, Y represents all the groups above when m is 0 to 4 and, in addition, Y represents —$CHOR^5$—; —O—; —NR^5CONR^5—; —NR^5CO—; —NR^5—; —$OCONR^5$—; —$NR^5C(NR^5)NR^5$—; —NR^5CSNR^5; —NR^5CS— or —NR^5SO_2—; —$NR^5C(O)O$—; or —$CSNR^5$—;

each R^5 independently represents hydrogen, alkyl or benzyl;

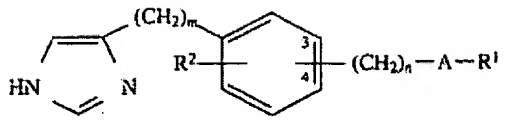
R^6 represents aryl, heteroaryl, or a 3- to 7-membered heterocyclic group having one to three heteroatoms in the ring, wherein the heteroatoms are selected from N, S and O, and wherein said R^6 group is optionally substituted by one to three substituents as defined below;

when Y is —SO_2—, then R^6, in addition to the above groups, also represents alkyl having 1 to 7 carbon atoms or a group —$NR^{10}R^{11}$ wherein R^{10} and R^{11} are independently selected from H, alkyl or trihalomethyl;

each R^1 is independently hydrogen, alkyl or trihalomethyl;

each R^7 is independently selected from hydrogen, alkyl, trihalomethyl, phenyl or benzyl, , wherein said phenyl and benzyl are optionally substituted by one to three substituents independently selected from of alkyl, halogen, trihalomethyl, CN, NO_2, OR^{10} or $NR^{10}R^{11}$, wherein R^{10} and R^{11} are as above defined.

U.S. Patent No. 5,578,616 discloses compounds comprising the following structural formula:



I

wherein:

A is selected from —O—CO—NR^1—, —CO—, —NR^1—CO—NR^1—, —NR^1—CO—, —NR^1—, —O—, —CO—NR^1—, —CO—O—, and —C(:NR^1)—NR^1—;

the groups R^1, which may be the same or different when there are two or three such groups in the molecule of formula I, are selected from hydrogen, and lower alkyl, aryl, cycloalkyl, heterocyclic and heterocyclyl-alkyl groups, and groups of the formula —(CH$_2$)$_y$—G, where G is selected from CO$_2$R^3, COR^3, CONR^{3}R^4, OR^3, SR^3, NR^{3}R^4, heteroaryl and phenyl, which phenyl is optionally substituted by halogen, lower alkoxy or polyhaloloweralkyl, and y is an integer from 1 to 3;

R^2 is selected from hydrogen and halogen atoms, and alkyl, alkenyl, alkynyl and trifluoromethyl groups, and groups of the formula OR^3, SR^3 and NR^{3}R^4;

R^3 and R^4 are independently selected from hydrogen, and lower alkyl and cycloalkyl groups, or R^3 and R^4 together with the intervening nitrogen atom can form a saturated ring containing 4 to 6 carbon atoms that can be substituted with one or two lower alkyl groups;

with the proviso that, when y is 1 and G is OR^3, SR^3 or NR^{3}R^4, then neither R^3 nor R^4 is hydrogen;

the group —(CH$_2$)$_n$—A—R^1 is at the 3- or 4-position, and the group R^2 is at any free position;

m is an integer from 1 to 3;

and n is 0 or an integer from 1 to 3;

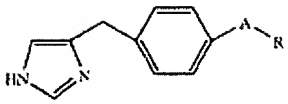
or a pharmaceutically acceptable acid addition salt thereof;

or a pharmaceutically acceptable salt thereof with a base when G is CO$_2$H;

including a tautomeric form thereof.

U.S. Patent No. 5,990,147 discloses compounds comprising the following structural formula:

(I)

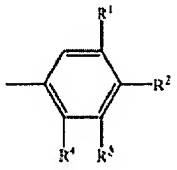


or a pharmaceutically acceptable acid addition salt or solvate thereof (or tautomer thereof, wherein:

A is $-CH_2-NH-CO-NH-$; $-CH_2-O-CO-NH-$ or $-CH_2CH_2-CO-NH-(CH_2)_m-$;

m is 0, 1 or 2;

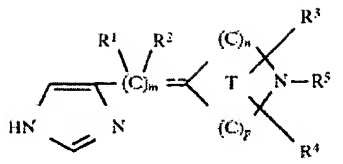
R is the group



wherein at least two of R^1, R^2, R^3 and R^4 are hydrogen and the two others are independently selected from H, halogen (e.g., Br, I, F, or Cl), CH_3, CF_3, OCH_3, OCF_3 or CN; and

with the proviso, that when A is $-CH_2-O-CO-NH-$ and R^1, R^3 and R^4 are all hydrogen, then R^2 can not be Cl.

U.S. Patent No. 5,807,872 discloses compounds comprising the following structural formula:



(1.0)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

(A) m is an integer selected from the group consisting of: 1 and 2;

(B) n and p are integers and are each independently selected from the group consisting of: 0, 1, 2, 3, and 4 such that the sum of n and p is 4 and T is a 6-membered ring;

(C) R^3 and R^4 are each independently bound to the same or different carbon atom of ring T such that there is only one R^3 group and one R^4 group in ring T, and each R^1, R^2, R^3, and R^4 is independently selected from the group consisting of:

(1) H;

(2) C_1 to C_6 alkyl; and

(3) $—(CH_2)_q—R^6$ wherein q is an integer of: 1 to 7, and R^6 is selected from the group consisting of: phenyl, substituted phenyl, $—OR^7$, $—C(O)OR^7$, $—C(O)R^7$, $—OC(O)R^7$, $—C(O)NR^7R^8$, CN and $—SR^7$ wherein R^7 and R^8 are as defined below, and wherein the substituents on said substituted phenyl are each independently selected from the group consisting of: —OH, —O—(C_1 to C_6)alkyl, halogen, C_1 to C_6 alkyl, $—CF_3$, —CN, and $—NO_2$, and wherein said substituted phenyl contains from 1 to 3 substituents;

(D) R^5 is selected from the group consisting of:

(1) H;

(2) C_1 to C_{20} alkyl;

(3) C_3 to C_6 cycloalkyl;

(4) $—C(O)OR^{7'}$; wherein $R^{7'}$ is the same as R^7 defined below except that $R^{7'}$ is not H;

(5) $—C(O)R^7$;

(6) $—C(O)NR^7R^8$;

(7) allyl;

(8) propargyl; and

(9) $—(CH_2)_q—R^6$, wherein q and R^6 are as defined above, and when q is equal to 1, then R^6 is not OH or SH;

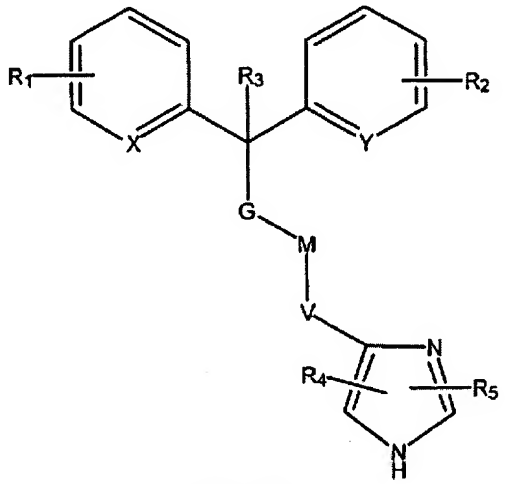
(E) R^7 and R^8 are each independently selected from the group consisting of: H, C_1 to C_6 alkyl, and C_3 to C_6 cycloalkyl;

(F) the dotted line (------) represents a double bond that is optionally present when m is 1, and n is not 0, and p is not 0 (i.e., the nitrogen in the ring is not bound directly to the carbon atom bearing the double bond), and when said double bond is present then R^2 is absent; and

(G) when m is 2, each R^1 is the same or different substituent for each m, and each R^2 is the same or different substituent for each m, and at least two of the substituents R^1 and/or R^2 are H.

Those skilled in the art will appreciate that the total number of substituents on each of the —(C)$_n$— and —(C)$_p$— groups is two, and that such substituents are independently selected from the group consisting of hydrogen, R^3 and R^4, such that there is a total of only one R^3 and one R^4 substituent in ring T.

The following PCT Publication discloses H3 antagonists and H1/H3 dual antagonists which may be used in the present invention: PCT Publication No. WO 02/24658 discloses compounds comprising the following formula:

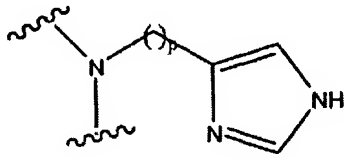


Formula I

wherein

G is selected from the group consisting of C_1-C_6 alkyl or a bond;

M is a moiety selected from the group consisting of –C=C-, -C≡C-, -C(=NR^7)-NR^6-, -NR^6-C(=NR^7)-, -NR^6-C(O)-NR^6-, -NR^6-C(O)-O-, -O-C(O)-NR^6-, -NR^6-C(O)-, -C(O)-NR^6-, -O-, -NR^6-, -C(O)-, -$N^+R^6R^8$-, and



p is 1 - 6

V is C_1-C_6 alkyl;

X and Y may be the same or different and are independently selected from the group consisting of N, CH, or N-oxide, with the proviso that at least one of X and Y is N or N-oxide;

R^1 and R^2 may each number 1-4 and are independently selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halogen, polyhalolower alkyl, -OH, -$N(R^6)_2$, -NO_2, -CN, -$COOR^6$, -$CONR^6R^8$, and

$-NR^6-C(O)-R^7$(wherein R^7 is not –OH or –CN);

R^3 is selected from hydrogen, lower alkyl, lower alkoxy, hydroxyl, polyhalolower alkyl, and a bond forming a double bond towards the moiety G when G is $C_1 - C_6$ alkyl;

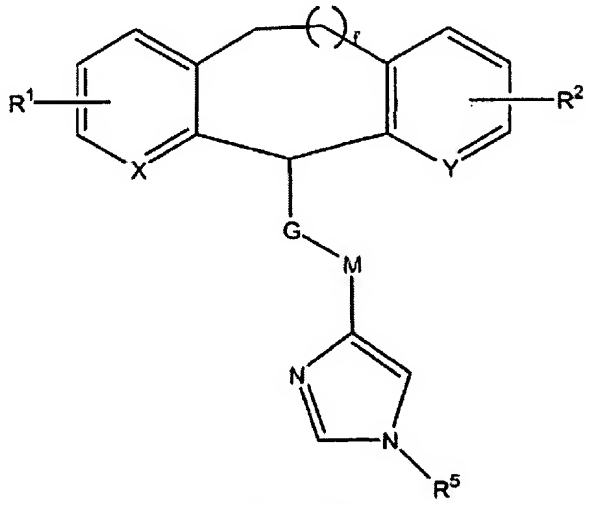
R^4 and R^5 are independently selected from the group consisting of hydrogen, lower alkyl, and polyhalolower alkyl;

R^6 and R^8 are independently selected from hydrogen, lower alkyl, aralkyl, alkylaryl, polyhalolower alkyl, substituted or unsubstituted phenyl; and substituted or unsubstituted benzyl; and

R^7 is selected from H, OH, alkoxy, cyano, phenyl, substituted phenyl, benzyl, and substituted benzyl;

with the proviso that when G is a bond and when M is either -O- or $-O-C(O)-NR^6-$, then one of X and Y is N; and with the further proviso that when R^3 is –OH or alkoxyl, and G is a bond, then $M \neq O$ or NR^6.

PCT Publication No. WO 02/24659 discloses compounds comprising the following structural formula:



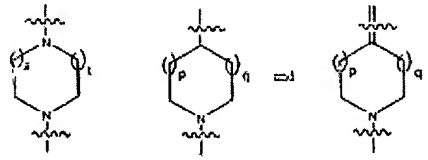
Formula I

wherein:

f = 0, 1 or 2;

X and Y are independently selected from the group consisting of N, CH or N-oxide;

G is a moiety selected from the group consisting of the moieties II, III and IV with the top end of said II, III and IV being linked to the tricyclic moiety and the bottom end of II, III and IV being linked to M:



II III IV

where e = t = 1 or 2; and p = q = 0, 1 or 2;

M is a moiety selected from the group consisting of C_1-C_6 alkyl; $-C(O)-(CH_2)_n-$; $-(CH_2)_x-A-(CH_2)_y-$; $-C(O)-O-(CH_2)_r-$; and $-C(O)-NR^3-(CH_2)_d-$; where A=O, S(O)$_r$-, and $-NR^4-$;

n= 0, 1, 2 or 3;

x is a whole number in the range 2-5;

y is a whole number in the range 0-5;

d is a number in the range 0-5;

r= 0, 1 or 2;

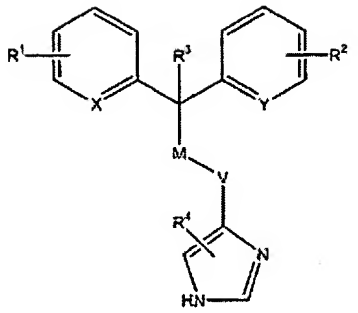
R^1 and R^2 may each number 1-3 and are independently selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halogen, OCF_3, $OCHF_2$, -OH, and $-N(R^4)_2$;

R^3 is selected from the group consisting of hydrogen, lower alkyl, and polyhaloloweralkyl;

R^4 is selected from hydrogen, lower alkyl, polyhalolower alkyl; and

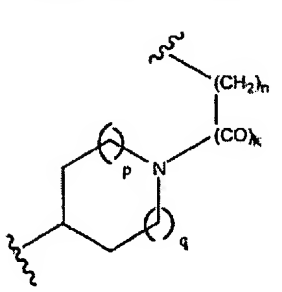
R^5 is H, C_1-C_6 alkyl or OH.

PCT Publication No. WO 02/44141 discloses compounds comprising the following structural formula:

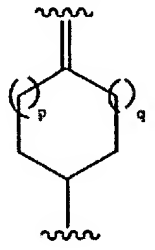


Formula I

M is a moiety having a general structure shown in Formula II or III:



II



III

where k = 0 or 1, n = 0-5, and p = q = 0, 1 or 2 with the proviso that when M is Formula III, R^3 is absent;

V is a moiety selected from the group consisting of C_1-C_8 alkyl; $-(CH_2)_x-A-(CH_2)_y-$; and $-(CH_2)_c-A-(CH_2)_m-C(O)-N(R^7)-(CH_2)_d-$, where A is -O-, $-S(O)_r-$, and $-NR^7-$; m = 0, 1, 2 or 3; x is a whole number in the range 2-8; y is a whole number in the range 1-5; c is a whole number in the range 2-4; and r= 0, 1 or 2; d is a number in the range 0-5;

X and Y are independently selected from the group consisting of N, CH, and N(O);

Z is selected from the group consisting of N, CH and N(O);

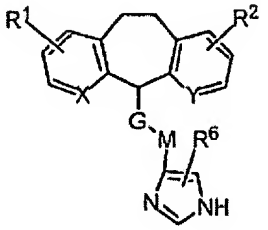
R^1 and R^2 may each number 1-4 and are independently selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halogen, polyhalolower alkyl, polyhalolower alkoxy, -OH, CN, NO_2, or $COOR^8$;

R^3 is selected from hydrogen, lower alkyl, lower alkoxy, hydroxyl, with the proviso that when n and k are both 0, then R^3 is not -OH or alkoxy;

R^4 is selected from the group consisting of hydrogen, lower alkyl, polyhalolower alkyl or -OH; and

R^7 and R^8 are independently selected from hydrogen, lower alkyl, substituted or unsubstituted phenyl; and substituted or unsubstituted benzyl.

PCT Publication No. WO 02/24657 discloses compounds comprising the following structural formula:



Formula 1

wherein

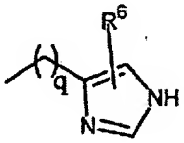
G is selected from the group consisting of $-(CH_2)_v-NR^3-$, $-(CH_2)_v-O-$, $-(CH_2)_v-S(O)_z-$, $-(CH_2)_v-NR^3-C(NR^4)-NR^3-$, $-(CH_2)_v-O-C(O)NR^3-$, $-(CH_2)_v-NR^3C(O)NR^3-$, $-(CH_2)_v-NR^3C(O)O-$, $-(CH_2)_v-NR^3C(O)-$, $-(CH_2)_vC(O)NR^3-$;

M is a branched or unbranched alkyl group consisting of 1-6 carbon atoms, or a branched or unbranched alkenyl group consisting of 2-6 carbon atoms;

X and Y are independently selected from the group consisting of N, CH or N-oxide;

R^1 and R^2 may each number 1-4 and are independently selected from the group consisting of H, halogen, lower alkyl, lower alkoxy, polyhalo lower alkoxy, OH, CF_3, NH_2, NHC(O)alkyl, CN or NO_2;

R^3 is independently selected from the group consisting of H, lower alkyl, substituted or unsubstituted phenyl, substituted or unsubstituted benzyl, or a group of the formula:



R^4 is selected from the group consisting of H, CN, CO_2R^5;

R^5 is selected from the group consisting of lower alkyl and substituted or unsubstituted benzyl;

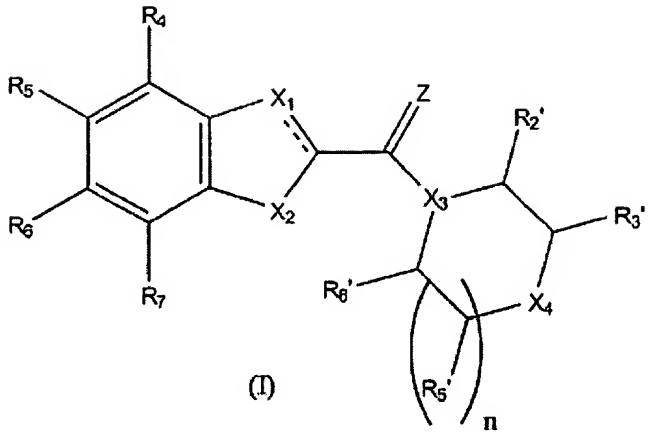
R^6 is selected from the group consisting of H or lower alkyl;

q is 2-5;

v is 0-6; and

z is 0, 1 or 2.

PCT Publication No. WO 02/72548 discloses compounds comprising the following structural formula:



(I)

Wherein R_1 is R_a, R_aR_b-, R_a-O-R_b-, or $(R_c)(R_d)$N-R_b-, where R_a is H, cyano, -(C=O)N(R_c)(R_d), -C(=NH)(NH_2), C_{1-10} alkyl, C_{3-8} alkenyl, C_{3-8} cycloalkyl, C_{2-5} heterocyclic radical, or phenyl; where R_b is C_{1-8} alkylene, C_{2-8} alkenylene, C_{3-8} cycloalkylene, bivalent C_{3-8} heterocyclic radical, or phenylene; and R_c and R_d are each independently H, C_{1-8} alkyl, C_{2-8} alkenyl, C_{3-8} cycloalkyl, or phenyl;

R_2' is H, methyl, ethyl, NR_pR_q, -(CO)NR_pR_q, -(CO)OR_r , -$CH_2NR_pR_q$, or CH_2OR_r; where R_p, R_q, and R_r are independently selected from C_{1-6} alkyl, C_{3-6} cycloalkyl, phenyl; (C_{3-6} cycloalkyl)(C_{1-2} alkylene), benzyl or phenethyl; or R_p and R_q taken together with the nitrogen to which they are attached, form a 4-7 membered heterocyclic ring with 0 or 1 additional heteroatoms selected from O, S, and N;

R_3' is H, methyl, ethyl, NR_sR_t, -(CO)NR_sR_t, -(CO)OR_u , -$CH_2NR_sR_t$, or CH_2OR_u; where R_s, R_t, and R_u are independently selected from C_{1-6} alkyl, C_{3-6} cycloalkyl, phenyl; (C_{3-6} cycloalkyl)(C_{1-2} alkylene), benzyl or phenethyl; or R_s and R_t taken together with the nitrogen to which they are attached, form a 4-7 membered heterocyclic ring with 0 or 1 additional heteroatoms selected from O, S, and N;

R_5' is methyl, ethyl, or H;

R_6' is methyl, ethyl, or H;

R_7' is methyl, ethyl, or H;

X_4 is NR_1 or S;

X_1 is CR_3;

R_3 is F, Cl, Br, CHO, R_f, R_fR_g-, R_f-O-R_g-, or $(R_h)(R_i)$N-R_g-, where R_f is H, C $_{1-6}$ alkyl, C $_{2-6}$ alkenyl, C $_{3-6}$ cycloalkyl, C $_{2-5}$ heterocyclic radical, or phenyl; where R_g is C $_{1-6}$ alkylene, C $_{2-6}$ alkenylene, C $_{3-6}$ cycloalkylene, bivalent C $_{3-6}$ heterocyclic radical, or phenylene; and R_h and R_i are each independently H, C $_{1-6}$ alkyl, C $_{2-6}$ alkenyl, C $_{3-6}$ cycloalkyl, or phenyl;

X_2 is NR_e or O, provided that X_2 is NR_e where X_1 is N; R_e is H or C $_{1-6}$ alkyl;

X_3 is N;

Z is =O or =S;

each of R_4 and R_8 is independently H, F, Cl, Br, I, COOH, OH, nitro, amino, cyano, C $_{1-4}$ alkoxy, or C $_{1-4}$ alkyl;

R_5 is H, F, Cl, Br, I, $(C=O)R_j$, OH, nitro, NR_jR_k, cyano, phenyl, -OCH_2-Ph, C $_{1-4}$ alkoxy, or C $_{1-4}$ alkyl;

R_7 is H, F, Cl, Br, I, $(C=O)R_m$, OH, nitro, NR_lR_m, cyano, phenyl, -OCH_2-Ph C $_{1-4}$ alkoxy, or C $_{1-4}$ alkyl;

wherein each of R_j, R_k, R_l, and R_m is independently selected from H, C_{1-6} alkyl, hydroxy, phenyl, benzyl, phenethyl, and C_{1-6} alkoxy;

each of the above hydrocarbyl (including alkyl, alkoxy, phenyl, benzyl, cycloalkyl, and so on) or heterocyclic groups being independently and optionally substituted with between 1 and 3 substituents selected from C $_{1-3}$ alkyl, halo, hydroxy, amino, and C $_{1-3}$ alkoxy;

wherein n is 0, 1, or 2; where n is 2, the moiety $-(CHR_{5'})_{n=2}$- is $-(CHR_{5'}$-$CHR_{7'})$- where $CHR_{5'}$ is between $CHR_{6'}$ and $CHR_{7'}$;

provided at least one of R_1, $R_{2'}$, R_3, R_4, R_5, R_6, and R_7 is other than H when Z is O;

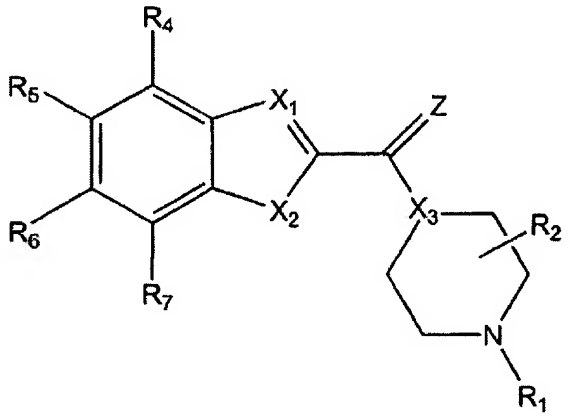
and provided, where Z is O, n =1, and each of R_4, R_5, R_6, R_7, $R_{2'}$, $R_{3'}$, $R_{5'}$, and $R_{6'}$ is H, (or at least 7, 8, or 9 of these 10 limitations apply) then (a) where X_2 is NH, then R_1 is (i) not methyl, pyridyl, phenyl, or benzyl, or (ii) is selected from the disclosed possibilities, but not C $_{1-2}$ alkyl and not a six-membered aryl or six-membered nitrogen-containing heteroaryl, or phenyl(C $_{1-2}$ alkylene) (alternatively, provided, where Z is O, n =1, and X_2 is NH, then at

least two (or three) of R_4, R_5, R_6, R_7, $R_{2'}$, $R_{3'}$, $R_{5'}$, and $R_{6'}$ is other than H); and (b) where X_2 is O, then R_1 is not methyl;

and provided, where Z is O, X_2 is NH, n = 1, R_1 is methyl, each of R_4, R_6, R_7, $R_{2'}$, $R_{3'}$, $R_{5'}$, and $R_{6'}$ is H (or at least 7, 8, 9, or 10 of these 11 limitations apply) , then R_5 is (i) not methoxy, (ii) not methoxy, or ethoxy, (iii) not C_{1-4} alkoxy, or (iv) not methoxy or hydroxy;

or a pharmaceutically acceptable salt, ester, or amide thereof.

According to one aspect of the invention, the invention features compounds of the following formula (Ib):



(Ib)

Wherein R_1 is R_a, R_aR_b-, R_a-O-R_b-, or $(R_c)(R_d)$N-R_b-, where R_a is H, C_{1-10} alkyl, C_{3-8} alkenyl, C_{3-8} cycloalkyl, C_{2-5} heterocyclic radical, or phenyl; where R_b is C_{1-8} alkylene, C_{3-8} alkenylene, C_{3-8} cycloalkylene, bivalent C_{3-8} heterocyclic radical, or phenylene; and R_c and R_d are each independently H, C_{1-8} alkyl, C_{3-8} alkenyl, C_{3-8} cycloalkyl, or phenyl;

R_2 is ortho (like $R_{2'}$ in formula (I)) or meta (like $R_{3'}$ in formula (I)), and is methyl or H;

X_1 is CR_3;

R_3 is F, Cl, Br, R_f, R_fR_g-, R_f-O-R_g-, or $(R_h)(R_i)$N-R_g-, where R_f is H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{3-6} cycloalkyl, C_{2-5} heterocyclic radical, or phenyl; where R_g is C_{1-6} alkylene, C_{2-6} alkenylene, C_{3-6} cycloalkylene, bivalent C_{3-6} heterocyclic radical, or phenylene; and R_h and R_i are each independently H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{3-6} cycloalkyl, or phenyl;

X_2 is NR_e or O, provided that X_2 is NR_e when X_1 is N; R_e is H or C_{1-6} alkyl;

X_3 is N;

Z is =O or =S;

each of R_4 and R_6 is independently H, F, Cl, Br, I, COOH, OH, nitro, amino, cyano, C_{1-4} alkoxy, or C_{1-4} alkyl;

R_5 is H, F, Cl, Br, I, $(C=O)R_j$, OH, nitro, NR_jR_k, cyano, $-OCH_2$-Ph, C_{1-4} alkoxy, or C_{1-4} alkyl;

R_7 is H, F, Cl, Br, I, $(C=O)R_m$, OH, nitro, NR_lR_m, cyano, C_{1-4} alkoxy, or C_{1-4} alkyl;

wherein each of R_j, R_k, R_l, and R_m is independently selected from H, C_{1-6} alkyl, hydroxy, and C_{1-6} alkoxy; and

each of the above hydrocarbyl or heterocyclic groups being independently and optionally substituted with between 1 and 3 substituents selected from C_{1-3} alkyl, halo, hydroxy, amino, and C_{1-3} alkoxy;

provided at least one of R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is other than H when Z is =O;

or a pharmaceutically acceptable salt, ester or amide thereof.

The present invention comprises compositions comprising an antagonist or a combination of antagonists which antagonize the H3 receptor (*e.g.*, any of the H3 antagonists mentioned herein), the H4 receptor (*e.g.*, any of the H4 antagonists mentioned herein), and, optionally, the H1 receptor (*e.g.*, any of the H1 antagonists mentioned herein) and pharmaceutical compositions thereof.

The ability of any substance to bind to a histamine receptor can be evaluated by using the methods set forth herein (*e.g.*, Examples) or by using the examples set forth in U.S. Patent No. 6,204,017.

Other antagonists may be, for example, small molecules, nucleic acids (*e.g.*, antisense oligonucleotides which bind to H1, H3 or H4 histamine receptor mRNA), peptides, or antibodies (and antigen-binding fragments thereof) which bind specifically to an H1, H3 or H4 receptor.

Pharmaceutical Compositions, Dosage and Administration

The present invention also includes a pharmaceutical composition comprising a histamine H3 receptor antagonist, a histamine H4 receptor antagonist and, optionally, a

histamine H1 receptor antagonist along with a pharmaceutically acceptable carrier along with methods for administrating the compositions to treat allergic conditions. The pharmaceutical compositions may be prepared by any methods well known in the art of pharmacy; see, *e.g.*, Gilman *et al.* (eds.) (1990), The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, *supra*, Easton, Penn.; Avis *et al.* (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications Dekker, New York; Lieberman *et al.* (eds.) (1990) Pharmaceutical Dosage Forms: Tablets Dekker, New York; and Lieberman *et al.* (eds.) (1990), Pharmaceutical Dosage Forms: Disperse Systems Dekker, New York.

Pharmaceutical compositions containing the antagonists can be prepared using conventional pharmaceutically acceptable excipients and additives and conventional techniques. Such pharmaceutically acceptable excipients and additives include non-toxic compatible fillers, binders, disintegrants, buffers, preservatives, anti-oxidants, lubricants, flavorings, thickeners, coloring agents, emulsifiers and the like. All routes of administration are contemplated including, but not limited to, parenteral (*e.g.*, subcutaneous, intramuscular, intraperitoneal, intravenous), and non-parenteral (*e.g.*, topical, ocular, transdermal, sublingual, inhalation, rectal, oral).

Unit forms of administration include oral forms such as tablets, capsules, powders, cachets, granules and solutions or suspensions, sublingual and buccal forms of administration, aerosols, implants, subcutaneous, intramuscular, intravenous, intranasal, intraocular, subcutaneous or rectal forms of administration.

When a solid composition is prepared in the form of tablets, *e.g.*, a wetting agent such as sodium lauryl sulfate can be added to micronized or non-micronized antagonists and mixed with a pharmaceutical vehicle such as silica, gelatin starch, lactose, magnesium stearate, talc, gum arabic or the like. The tablets can be coated with sucrose, various polymers, or other appropriate substances. Tablets can be treated so as to have a prolonged or delayed activity and so as to release a predetermined amount of active principle continuously or at predetermined intervals, *e.g.*, by using ionic resins and the like.

A preparation in the form of gelatin capsules may be obtained, *e.g.*, by mixing the antagonists with a diluent, such as a glycol or a glycerol ester, and incorporating the resulting mixture into soft or hard gelatin capsules.

A preparation in the form of a syrup or elixir can contain the antagonists together, *e.g.*, with a sweetener, methylparaben and propylparaben as antiseptics, flavoring agents and an appropriate color.

Water-dispersible powders or granules can contain the antagonists mixed, *e.g.*, with dispersants, wetting agents or suspending agents, such as polyvinylpyrrolidone, as well as with sweeteners and/or other flavoring agents.

Rectal administration may be provided by using suppositories which may be prepared, *e.g.*, with binders melting at the rectal temperature, for example cocoa butter or polyethylene glycols.

Parenteral, intranasal or intraocular administration may be provided by using, *e.g.*, aqueous suspensions, isotonic saline solutions or sterile and injectable solutions containing pharmacologically compatible dispersants and/or solubilizers, for example, propylene glycol or polyethylene glycol.

Thus, to prepare an aqueous solution for intravenous injection, it is possible to use a co-solvent, *e.g.*, an alcohol such as ethanol or a glycol such as polyethylene glycol or propylene glycol, and a hydrophilic surfactant such as Tween® 80. An oily, intramuscular injectable solution can be prepared, *e.g.*, by solubilizing the antagonists with a triglyceride or a glycerol ester.

Topical administration can be provided by using, *e.g.*, creams, ointments or gels.

Transdermal administration can be provided by using patches in the form of a multilaminate, or with a reservoir, containing the antagonists and an appropriate solvent.

Administration by inhalation can be provided by using, *e.g.*, an aerosol containing sorbitan trioleate or oleic acid, for example, together with trichlorofluoromethane, dichlorofluoromethane, dichlorotetrafluoroethane or any other biologically compatible propellant gas; it is also possible to use a system containing the antagonists, by themselves or associated with an excipient, in powder form.

The antagonists can also be formulated as microcapsules or microspheres, *e.g.*, liposomes, optionally with one or more carriers or additives.

Implants are among the prolonged release forms which can be used in the case of chronic treatments. They can be prepared in the form of an oily suspension or in the form of a suspension of microspheres in an isotonic medium.

The daily dose of a antagonists can be determined by a clinician and is generally dependent on the potency of the compound administered, the age, weight, condition and response of the subject.

Methods of the present invention may include administration of the antagonists along with, for example, known antihistamine, decongestant or anti-allergy agents. The administration and dosage of such agents is typically as according to the schedule listed in the product information sheet of the approved agents, in the Physicians' Desk Reference

2003 (Physicians' Desk Reference, 57th Ed); Medical Economics Company; ISBN: 1563634457; 57th edition (November 2002), as well as therapeutic protocols well known in the art. For example, histamine antagonists of the present invention can be administered to a patient at a "therapeutically effective dosage". A therapeutically effective dosage is any dosage which is sufficient to alleviate or prevent the symptoms or physiological effects of allergic or non-allergic airway obstruction including, but not limited to, allergic rhinitis, congestion (*e.g.*, sinus congestion), pulmonary inflammation, acute respiratory distress syndrome, asthma, bronchitis, chronic obstructive pulmonary disease, pulmonary fibrosis, emphysema, respiratory infections and sinus infections to any degree. In one embodiment of the invention, a histamine receptor antagonist of the present invention is administered to a patient or subject in need of such treatment (*e.g.*, a patient or subject suffering from or susceptible to any of the indications mentioned herein) at a dosage of about 5 to about 2000 mg per day or about 50 mg per day to about 1900 mg/day or about 100 mg per day to about 1800 mg/day or about 300 mg per day to about 1600 mg/day or about 500 mg per day to about 1200 mg/day or about 750 mg per day to about 1000 mg/day or about 5 mg per day to about 500 mg per day or about 500 mg per day to about 1000 mg per day or about 1000 mg per day to about 2000 mg per day.

Typical agents which may be included along with the histamine receptor antagonists include glucocorticoids (*e.g.*, mometasone, fluticasone, budesonide), Non-steroidal anti-inflammatory drugs (NSAIDs) (*e.g.*, COX2 inhibitors (*e.g*, rofecoxib, celecoxib) ibuprofen, naproxen), leukotriene receptor antagonists (*e.g.*, montelukast sodium), M3 antagonists (*e.g.*, ipratropium, tiotropium) and antibiotics (*e.g.*, penicillin, amoxicillin, ampicillin, methicillin).

The histamine receptor antagonists of the invention along with any additional agents (discussed above) may be formulated together into a single composition or into two or more separate compositions for simultaneous consumption. Alternatively, for example, an H1 antagonists may be administered to a subject at a different time than when the H3 and H4 antagonists are administered; for example, each administration may be given non-simultaneously at several intervals over a given period of time.

Indications

The compositions of the present invention can be used to treat or prevent medical conditions characterized by allergic or non-allergic airway obstruction including, but not limited to, allergic rhinitis, congestion (*e.g.*, sinus congestion), pulmonary inflammation,

acute respiratory distress syndrome, asthma, bronchitis, chronic obstructive pulmonary disease, pulmonary fibrosis, emphysema, respiratory infections and sinus infections.

Clinical symptoms of seasonal allergic rhinitis typically include nasal itching and irritation, sneezing and watery rhinorrhea, frequently accompanied by nasal congestion. The perennial allergic rhinitis clinical symptoms are similar, except that nasal blockage may be more pronounced. Either type of allergic rhinitis may also cause other symptoms such as itching of the throat and/or eyes, epiphora and edema around the eyes. These symptoms may vary in intensity from the nuisance level to debilitating. Other types of rhinitis present similar symptoms. In addition to other processes, allergic rhinitis involves the release of histamine (*e.g.*, from mast cells) which is a mediator in immediate hypersensitivity reactions.

Congestion, particularly sinus congestion, involves blockage of one or more of the four pairs of sinus passageways in the skull. The blockage may result from inflammation and swelling of the nasal tissues or from secretion of mucus. It may be acute or chronic. Acute sinus congestion is most often caused by the common cold. Chronic sinus congestion may result from environmental irritants such as tobacco smoke, food allergens, inhaled allergens, or foreign bodies in the nose. Sinus congestion leads to impaired flow of fluids in the sinuses, which predisposes individuals to bacterial infections that can cause sinusitis.

Pulmonary inflammation is a condition which is often characterized by wheezing and shortness of breath. When the lungs are exposed to allergens (*e.g.*, particulate matter, automobile exhaust or pollen) and pathogens (*e.g.*, *Pseudomonas aeruginosa*) pulmonary inflammation often occurs.

Adult (acute) respiratory distress syndrome (ARDS) is a condition characterized by pulmonary inflammation. In general, ARDS results in the rapid onset of progressive malfunction of the lungs, especially with regard to the ability to take in oxygen, usually associated with the malfunction of other organs. The condition is associated with extensive pulmonary inflammation and small blood vessel injury in all affected organs.

The fundamental pathophysiologic entity resulting in the clinical disease of asthma is airway inflammation. Histological findings in the asthmatic airway may include bronchial occlusion with mucous and cellular debris, denudation of the epithelial layer, edema and inflammatory infiltrate in the submucosa, mucous gland hypertrophy, and bronchial smooth muscle hypertrophy.

The methods and compositions of the present invention may also be used to treat chronic bronchitis, chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, emphysema and sinus and respiratory infections.

Kits

The present invention also provides kits comprising the components of the combinations of the invention in kit form. A kit of the present invention includes one or more components including, but not limited to, one or more histamine H3 antagonists, for example, as discussed herein, in association with one or more histamine H4 receptor antagonists, for example, as discussed herein and, optionally, in association with one or more histamine H1 receptor, for example, as discussed herein. The antagonists can be formulated as a pure composition or in combination with a pharmaceutically acceptable carrier, in a pharmaceutical composition.

In one embodiment, a kit includes one or more histamine H3 antagonists, or a pharmaceutical composition thereof, in one container (*e.g.*, in a sterile glass or plastic vial), one or more histamine H4 antagonists, or a pharmaceutical composition thereof, in another container (*e.g.*, in a sterile glass or plastic vial) and, optionally, one or more histamine H1 antagonists, or a pharmaceutical composition thereof, in another container (*e.g.*, in a sterile glass or plastic vial).

In another embodiment of the invention, the kit comprises a combination of the invention, including one or more histamine H3 antagonists along with one or more histamine H4 antagonists and, optionally, one or more histamine H1 antagonists formulated together, optionally, along with a pharmaceutically acceptable carrier, in a pharmaceutical composition, in a single, common container.

If the kit includes a pharmaceutical composition for parenteral administration to a subject, the kit can include a device for performing such administration. For example, the kit can include one or more hypodermic needles or other injection devices.

The kit can include a package insert including information concerning the pharmaceutical compositions and dosage forms in the kit. Generally, such information aids patients and physicians in using the enclosed pharmaceutical compositions and dosage forms effectively and safely. For example, the following information regarding a combination of the invention may be supplied in the insert: pharmacokinetics, pharmacodynamics, clinical studies, efficacy parameters, indications and usage, contraindications, warnings, precautions, adverse reactions, overdosage, proper dosage

and administration, how supplied, proper storage conditions, references, manufacturer/distributor information and patent information.

EXAMPLES

The following examples are provided to further describe the present invention and should not be construed to limit the scope of the present invention.

The following examples make reference to standard methods known to those skilled in the art which may be performed, as described, *e.g.*, in Maniatis, *et al.*, Molecular Cloning: A Laboratory Manual, 1982, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, (2d ed.), Vols 1-3, 1989, Cold Spring Harbor Press, NY; Ausubel, *et al.*, Biology, Greene Publishing Associates, Brooklyn, NY; or Ausubel, *et al.*, (1987 and Supplements), Current Protocols in Molecular Biology, Greene/Wiley, New York; Innis, *et al.*, (eds.) PCR Protocols: A Guide to Methods and Applications, 1990, Academic Press, N.Y.

Example 1. Screening Assays for Histamine H4 Receptor Antagonists.

In this example, the ability of several compounds to compete against radiolabeled histamine for binding to membrane-bound human histamine H4 receptor is evaluated. The histamine H4 receptor used in this assay is SP9144 which is set forth in SEQ ID NOs: 1 and 2 of U.S. Patent No. 6,204,017.

Membrane preparation. Forty-eight hours after transfection with plasmid containing the sequence for SP9144, HEK293 cells were harvested from T150 flasks by incubating 5 minutes in 5 ml of 5 mM EDTA/Hanks' balanced salt solution followed by repeated pipeting. They were centrifuged 5 minutes at 1000 X g. The EDTA/PBS was decanted and an equal volume of ice-cold 50 mM Tris-HCl, pH 7.5, was added and cells were broken up with a Polytron (PT10 tip, setting 5, 30 seconds). Nuclei and unbroken cells were sedimented at 1000 X g for 10 minutes and then the supernatant was centrifuged at 50,000 X g for 10 minutes. The supernatant was decanted, the pellet was resuspended by Polytron, a sample was taken for protein assay (bicinchoninic acid, Pierce; Rockford, IL), and the tissue was again centrifuged at 50,000 X g. Pellets were stored frozen at –20° C.

Binding assay. For saturation binding, four concentrations of [^{3}H]-histamine (15 Ci/mmol, Dupont NEN; Boston, MA) were incubated without and with 10^{-5} M histamine in triplicate with 50 µg of membrane protein in a total volume of 200 µl of 50 mM Tris-HCl, pH 7.5, for 30 minutes at 30° C. Samples were filtered on GF/B filters and washed

thrice with 2 ml of cold Tris buffer. Filters were dried in a microwave oven, impregnated with Meltilex wax scintillant, and counted at 45% efficiency.

Competition binding assays. Five concentrations of compounds were incubated in triplicate with 18 nM [^{3}H]-histamine and 70 µg of membrane protein under conditions as described above.

Curves were fit to the data with Prism (GraphPad Software) nonlinear least-squares curve-fitting program and K_i values were derived from IC_{50} values according to Cheng and Prusoff (Cheng, *et al.*, (1973) Biochem. Pharm. 22:3099-3108). The data generated in these experiments is shown, below, in Table 3.

Table 3. Compound Potencies Versus [^{3}H]Histamine Binding to SP9144-Transfected HEK293 Cells.

Compound	$K_i \pm SEM^1$ (nM)
Imetit	3.1 ± 0.7
Clobenpropit	7.2 ± 0.5
Histamine	9.7 ± 0.9
N^{α}-methylhistamine	63 ± 2
Burimamide	100 ± 10
(R)-α-methylhistamine	140 ± 10
Thioperamide	210 ± 50
Dimaprit	380 ± 70
(S)-α-methylhistamine	3400 ± 300
Chlorpheniramine	> 10 µM
Cimetidine	> 10 µM

[1]Standard error of the mean

The methods set forth in this example may be adapted to evaluate the ability of other substances to antagonize histamine H4 receptors.

Methods by which compounds can be evaluated to determine activity at histamine H3 receptors include the guinea pig brain membrane assay and the guinea pig neurogenic ileum contraction assay, both of which are described in U.S. Patent No. 5,352,707. Another useful assay utilizes rat brain membranes and is described by West, *et al.*, (1990) Molecular Pharmacology 38: 610-613.

A particularly useful screening assay measures binding to sites in guinea pig brain membranes. This test is described in detail by Korte, *et al.*, (1990) Biochem. Biophys. Res. Comm. 168: 979-986, and quantifies the inhibition of radiolabeled N^{α} - methylhistamine binding to tissues by candidate compounds.

Affinity values (K_i) may be determined using the following formula:

$K_i = IC_{50} / (1 + (\text{concentration of ligand} / \text{affinity } (K_D) \text{ of radioligand}))$

The method of Korte, *et al* (*supra*) was used to analyze thioperamide and clobenpropit. The results are set forth below in Table 4 (see also WO 98/06394):

Table 4. Affinities of THIO and CLOB for the Histamine H3 Receptor.

Compound	K_i (nM)
Thioperamide	12
Clobenpropit	0.1

Example 2. Screening Assay for Histamine H1, H3 and H4 Receptor Antagonists.

In the present example, the affinities of several compounds for the H1, H3 and H4 receptors was determined by a membrane binding assay.

Materials. Rat and guinea-pig brains were obtained frozen from Rockland Immunochemicals (Gilbertsville, PA). Cell lines expressing recombinant human receptors were generated by using standard transfection techniques. The following radioligands were obtained from Dupont NEN (Boston, MA): [^{3}H]-pyrilamine, 23 Ci/mmol, for H1 binding; [^{3}H]-N$^{\alpha}$-methylhistamine, 82 Ci/mmol, for H3 binding and [^{3}H]-histamine, 20 Ci/mmol, for H4 binding.

Methods. Recombinant cell lines (*i.e.*, human H1-CHO cells, human H3-HEK293 and human H4-HEK293 cells) were cultured in Dulbecco's modified Eagle's medium/10% fetal bovine serum supplemented with 2 mM glutamine, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified 5% CO_2 atmosphere at 37° C. Selection was maintained with 0.5 mg geneticin/ml. Cells were harvested for membrane preparation by aspirating media, replacing it with Hanks' balanced salt solution/5 mM EDTA, and incubating flasks for 10 minutes at 37° C. Cells were pelleted by centrifugation at 1000 X *g* for ten minutes at 4° C.

Membrane preparation. Membranes were prepared by disrupting cells or tissue in at least ten volumes of ice-cold 50 mM Tris-HCl, pH 7.5 at 25° C, with a Polytron. homogenates were centrifuged ten minutes at 1000 X *g* and the supernatants were then centrifuged for ten minutes at 50,000 X *g*. Pellets from this centrifugation step were resuspended with a Polytron, a sample was taken for protein determination (BCA; Pierce; Rockford, IL), and the resuspension was again centrifuged at 50,000 X *g*. Brain membranes were stored as pellets, cell membranes as suspensions of 1 mg protein/ml Tris buffer at -20° C.

Binding assays. Membrane (300 μg of brain membrane protein, 5-10 μg of recombinant cell membrane) was incubated with radioligand at a concentration near its K_D value without or with inhibitor compounds in a total volume of 200 μl Tris buffer. Nonspecific binding was determined in the presence of 10^{-6} M chlorpheniramine for H1 binding, 10^{-6} M clobenpropit for H3 binding, or 10^{-5} M thioperamide for H4 binding. Assay mixtures were incubated for 30 minutes at 30° C in polypropylene, 96-well, deep-well plates then filtered through 0.3% polyethylenimine-soaked GF/B filters. These were washed three times with 1.2 ml of Tris buffer, dried in a microwave oven, impregnated with Meltilex wax scintillant and counted at 40% efficiency in a Betaplate scintillation counter (Wallac). IC_{50} values were determined by interpolation or by nonlinear, least-squares, curve-fitting with the Prism program (GraphPad Software). K_i values were determined in the manner of Cheng and Prusoff (Cheng, *et al.*, (1973) Biochem. Pharm. 22:3099-3108).

The data generated in these experiments are shown, below, in Table 5.

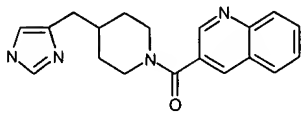
Table 5. Equilibrium Dissociation Constants for the Compounds of Formulas 18-36 at Histamine Receptors H1, H3 and H4.

Formula	H4 Ki (nM)	H3 Ki (nM)	H1 Ki (nM)
18	34	0.06	
19	38	55	0%
20	38	2	
21	90	3	
22	92	0.8	330
23	205	6	660
24	290	3	29
25	300	0.7	
26	320	15	0%
27	325	4	
28	390	3	2
29	440	19	
30	480	10	310
31	770	8	5
32	850	17	32%
33	870	470	
34	1100	19	48%
35	1100	7	0%
36	2%	1%	15

The methods in the foregoing examples can easily be adapted to determine whether any other substance binds to a histamine H1, H3 or H4 receptor.

Example 3. Effect of a Compound Comprising Formula 19 on BAL cells Recovered from LPS-Challenged Rats.

The following example demonstrates the ability of a compound comprising formula 19 (*i.e.*,



) to reduce the lipopolysaccharide (LPS)-induced inflammatory response in rat airways.

Male Sprague-Dawley rats (250-300 g) were anesthetized by inhalation of isoflurane (flow rate 1 ml/min; supplemented with O_2). Using a Penn-Centry microspray needle, 0.1 ml of a 100-μg/ml LPS solution in saline was injected into the trachea. Animals not challenged with the LPS solution received 0.1 ml of saline. Animals were placed on a heat pad until they recovered from anesthesia. Afterward, they were returned to their cages and allowed food and water *ad libitum*. All animals survived these manipulations and no additional interventions were required to ensure their survival. Animals fasted overnight were orally dosed with either the standard PD4 inhibitor and positive-control, SB207499 (c-4-cyano-4-(3-cyclopentyloxy-4-methoxy-phenyl)-r-1-cyclohexanecarboxylic acid; Barnette, *et al.*, (1998) J. Pharm. Exp. Ther. 284: 420-426), the compound of formula 17 or vehicle as a negative-control (0.4% methylcellulose) five hours before the LPS challenge.

At appropriate time points after intratracheal challenge with LPS, animals were surgically prepared with a tracheal cannula. Surgery was performed under anesthesia. The airways were flushed with 2 × 2 ml of 0.9% saline and the two washings pooled. Lavage fluid was centrifuged (350g, 4°C, 7 minutes), supernatant aspirated, erythrocytes lysed, and pellet washed in phosphate-buffered saline containing 10% heat-inactivated fetal calf serum and 10 μg/ml DNase I. The cell suspension was centrifuged, supernatant aspirated, and pellet resuspended in the same buffer. Total cell counts were performed using a Nebauer hemacytometer. Differential cell counts were conducted on Cytospin-prepared slides stained with Fisher's Leukostat stain. At least 200 cells were assessed per slide and standard morphological criteria were used to define neutrophilic cells.

The total number of cells and the number of neutrophils in the broncheoalveolar lavage (BAL) recovered from rats treated with the compound of formula 17, SB207499 or a blank were counted and compared. Fewer cells (*i.e.*, neutrophils or total cells) were counted in the BAL of LPS-challenged rats treated with the compound of formula 17 or with SB207499 than that of rats treated with a blank. The formula 17-dependent and SB207499-dependent inhibition of cellular influx into the BAL indicates that these compounds inhibit the pulmonary inflammation response induced by LPS. The cells

identified in the BAL were primarily neutrophils indicating that the inflammatory response induced by LPS was primarily a neutrophilic inflammatory response. The data from these experiments is set forth below in Tables 6 and 7.

Table 6. Inhibition of Total Cellular Influx into BAL in Response to LPS Challenge.

Treatment	% Inhibition
SB207499 (10 mg/kg)	61.2
Formula 19 (10 mg/kg)	64.5

Table 7. Inhibition of Neutrophilic Cellular Influx into BAL in Response to LPS Challenge.

Treatment	% Inhibition
SB207499 (10 mg/kg)	68.7
Formula 19 (10 mg/kg)	71.6

The experiments set forth in this example could easily be adapted to test the ability of any other compound or combination of compounds to inhibit pulmonary inflammation.

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The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Patents, patent applications, publications, product descriptions, and protocols are cited throughout this application, the disclosures of which are incorporated herein by reference in their entireties for all purposes.